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THE TRANSMISSION OF PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS*

I. Experimental Methods

COMMISSION ON ACUTE RESPIRATORY DISEASES**
Fort Bragg, North Carolina

* This investigation was supported in part through the Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, United States Army, and by grants from the Commonwealth Fund, the W. K. Kellogg Foundation, the John and Mary R. Markle Foundation, and the International Health Division of the Rockefeller Foundation to the Board for the Investigation and Control of Influenza and Other Epidemic Diseases for the Commission on Acute Respiratory Diseases.

** Members and professional associates of the Commission on Acute Respiratory Diseases are: John H. Dingle, Major, M.C., AU.S., Director; Theodore J. Abernethy, Major, M.C., A.U.S.; George F. Badger, Major, M.C., A.U.S.; J. W. Beard, M.D.; Norman L. Cressy, Major, M.C., A.U.S.; A. E. Feller, M.D.; Irving Gordon, M.D.; Alexander D. Langmuir, Major, M.C., A.U.S.; Charles H. Rammelkamp, M.D.; Elias Strauss, Captain, M.C., A.U.S.; Hugh Tatlock, Captain, M.C., A.U.S.

INTRODUCTION

In 1943 and 1944 studies of the transmission of primary atypical pneumonia to human volunteers were undertaken to determine whether or not (*1*) atypical pneumonia could be transmitted with secretions of the respiratory tract of patients, (*2*) the disease so produced resembled the naturally acquired infection, and (*3*) the illnesses could be induced by bacteria-free filtrates. Three experiments were conducted: the first, in October 1943; the second and third, during the summer of 1944.

The results of the first and preliminary experiment (1) indicated that primary atypical pneumonia was transmissible by means of untreated throat washings and sputa sprayed into the respiratory tract of human volunteers. Respiratory illnesses of variable clinical pattern developed in 10 of 12 men who were kept in group-isolation for a period of 6 weeks and observed daily for the development of any signs of illness. Similar illnesses did not occur in a group of un-inoculated individuals.

The second and third experiments comprised a continuation and extension of this earlier study. The number of volunteers was increased, rigid quarantine restrictions were employed and the infectivity of filtered as well as untreated and autoclaved respiratory tract secretions was tested. A summary of the results obtained in these two experiments, the second and third in the series, has been reported elsewhere (2, 3). The present communication deals with the experimental methods employed in these studies. Details concerning the results obtained and the clinical and laboratory features of experimentally induced illnesses are given in the papers which follow (4a, b, c.).

A. SECOND EXPERIMENT

Subjects. The subjects were all conscientious objectors who had volunteered for the project after the nature of the experiment had been thoroughly explained to them. Thirty-six men between the ages of 19 and 45 were selected after clinical and roentgenographic examina-

tion had shown them to be free from acute or chronic organic or infectious disease.

Locale. The experiments were performed in a hotel with facilities adequate for the isolation of the volunteers in individual rooms equipped with private bath. Sixteen men were quartered on the third floor and twenty men on the second floor.

Isolation Procedures. The volunteers were isolated in their rooms during the entire experimental period in order to prevent chance exposure to atypical pneumonia through outside contact. The usual techniques of a contagious hospital were instituted; gowns and masks were worn by the attending staff. Food was prepared in the hotel kitchen and distributed on individual compartment trays. Steam sterilization of dishes, glassware and silverware was accomplished after each meal.

Pre-Inoculation Studies. The volunteers were isolated for three weeks prior to inoculation since the available evidence suggested this interval as the probable maximum limit of the incubation period of atypical pneumonia. During this time, the temperature (oral), pulse and respiratory rates were recorded twice daily. A history and physical examination referable to respiratory symptoms and signs were performed every other day. Initial roentgenographic examinations of the chest included posterior-anterior stereoscopic views, right and left anterior oblique and right and left lateral views. In addition, three films of the accessory sinuses were taken in the conventional positions. Single posterior-anterior chest films were taken on the tenth and twenthieth days after the initial films. The radiographic equipment was a standard General Electric shock-proof unit equipped with 200 milliampere tube. All chest films were made with the subject in the upright position and at a distance of 72 inches; the exposure was from $\frac{1}{10}$ to $\frac{1}{20}$ second, using intensifying screens. Processing of all exposed films was accomplished at the hotel by the use of the Standard U. S. Army Portable Field Developing Unit.

Complete blood counts using venous blood were taken on each volunteer at the beginning of the experiment. Leukocyte and differential counts on blood obtained in similar manner, were repeated weekly; these studies were performed in the Regional Hospital Labora-

tory, Fort Bragg. The erythrocyte sedimentation rate was determined weekly by the Rourke-Ernstene method. Blood for serological study was also taken at weekly intervals. The blood was allowed to clot at room temperature and the serum was stored in rubber-capped sterile bottles at 4°C. Bacteriological study of the throat and nose flora was performed weekly. Swab cultures were always taken before breakfast, placed immediately in 5 ml. of broth and transported to the Commission Laboratory at Fort Bragg. The cultures were subsequently handled according to a standard technique (5).

Presence or absence of the following organisms on blood-agar plates was noted: Beta hemolytic streptococcus, *Staphylococcus aureus*, pneumococcus, *H. influenzae*, *H. hemolyticus*, and Friedlander's bacillus. The standard mouse technique was also employed for the detection of pneumococci. In addition, special cultural methods were utilized for the isolation of streptococcus MG (6).

Inoculum. The inoculum consisted of throat washings and sputum from seven patients with atypical pneumonia, admitted to the wards of the Regional Hospital, Fort Bragg, during the previous two months. Each of these patients was ill with the characteristic syndrome of primary atypical pneumonia (7), confirmed roentgenographically. The pertinent clinical and laboratory data obtained from these donors are given in Table 1. Immediately after collection, the specimens were placed in Pyrex test tubes, sealed with rubber stoppers, frozen in a carbon-dioxide ice-alcohol bath and then stored at −70°C.

Two days prior to inoculation the frozen specimens were thawed rapidly in a 37°C. water bath, and pooled in proportion of one part of sputum to five parts of throat washings. After thorough mixing in a Waring Blendor, approximately one-third of this pooled material was filtered through Corning sintered-glass or Seitz filters, and another third was autoclaved at 15 pounds pressure for ten minutes; the remainder was untreated. The three varieties of inoculum were placed in rubber-stoppered Pyrex Erlenmeyer flasks, refrozen in a carbon-dioxide ice-alcohol bath and stored at −70°C. until ready for use.

Aerobic and anaerobic cultures of the filtered and autoclaved material were sterile. Cultures of the untreated material on a variety of media showed the following organisms: alpha, alpha prime and gamma

streptococci, *Staphylococcus aureus*, *H. influenzae*, a coliform organism and a gram-positive micrococcus which were not further identified. Streptococcus MG, although cultured by special methods (6), was not isolated from any of the three types of inocula. Each of the individual specimens of sputum, as well as the pooled, untreated inoculum, was concentrated and examined for acid-fast bacilli by the Ziehl-Neelsen stain. No organisms morphologically resembling tubercle bacilli were found. Guinea pigs inoculated with these specimens remained well.

Inoculation Procedure. Prior to inoculation, each volunteer was arbitrarily assigned to one of three groups, A, B or C, in such a manner that concentration of any given group of men within a particular part of the hotel was avoided. The groups contained 12 men each; group A received filtered inoculum, group B, autoclaved material and group C, the same material untreated.

A group of rooms situated on the first floor of the hotel, as remote as possible from the volunteers, was utilized for the inoculation. Three separate rooms, on the same side of the hall, were employed for the three types of inocula. Sheets, saturated with cresol solution, were hung over the doors outside the rooms. The doors were opened frequently to permit entrance or exit of the volunteers and attending staff. Windows in the rooms were kept closed. The volunteers and professional personnel were masked and gowned.

A total of 10 ml. was administered to each man in three separate inoculations during the course of a single day (Table 2). The inoculum was given into the nose and throat, in synchronization with deep inspiration by means of an atomizer and nebulizer which were operated either manually or by a motor-driven air pump (the power-spray commonly employed by otolaryngologists). The individuals in each group, at one of the three inoculation sessions, received a small amount of inoculum by means of a medicine dropper. The equipment was sterilized by boiling between each set of inoculations; during any single session the external surfaces of the equipment were wiped off with alcohol. The inner surface of the tubing of the power spray was not sterilized.

The separate inoculations given each volunteer consumed approxi-

TABLE 1

Clinical and Laboratory Findings in Cases of Primary Atypical Pneumonia Selected as Donors of Specimens, Second Experiment

SPECIMEN					SERA		
Patients	Lobes involved	Type*	Day of disease	Cultural results†	Day of disease obtained	Cold hemagglutinin titer	Streptococcus MG agglutinin titer
H. J.	R. M.	Sp.	5	β Hemolytic streptococcus	3	16	<10
	R. L.			(group F); *H. hemolyticus*	7	16	<10
					15	16	<10
					22	8	<10
					36	<8	<10
R. V.	R. L.	Sp.	6	α Streptococcus	5	16	<10
	L. L.	Sp.	7	β Hemolytic streptococcus (type 19)	10	256	<10
		Sp.	8	α Streptococcus	17	256	40
		Sp.	10	Pneumococcus, type 28	25	128	80
J. J.	L. L.	Sp.	2	Pneumococcus, type 31; *H. influenzae*	2	<8	<10
		Sp.	3	Pneumococcus, type 31	10	64	40
		Sp.	5	No Growth	15	512	80
W. C.	L. L.	Sp.	5	Pneumococcus, type 3	3	<8	<10
					10	256	<10
					17	128	<10
					24	—‡	20
R. H.	R. L.	Sp.	3	Pneumococcus, type 20	3	64	<10
	L. L.	Sp.	5	No Growth	10	512	40
		Sp.	6	No Growth	17	512	—
					25	256	—
G. Y.	R. L.	Sp.	6	Pneumococcus, types, 11, 32; *H. influenzae*	4	<8	<10
		Sp.	7	Pneumococcus, type 11; *H. influenzae*	12	512	<10
		Sp.	8	α Streptococcus	33	64	—
		Sp.	9	α Streptococcus			
A. A.	R. M.	T. W.	4	α Streptococcus, *H. influenzae*	4	64	10
	R. L.	T. W.	5	*H. influenzae*	11	4096	20
		T. W.	6	*Staphylococcus aureus.*§ *H. influenzae; H. hemolyticus*	18	8192	40
		T. W.	7	*Staphylococcus aureus.*§ *H. influenzae*	33	1024	—
					40	1024	—

* Sp. = sputum; T. W. = throat washings.

† Results given for individual specimens.

‡ Test not done.

§ Non-hemolytic, coagulase negative.

mately 30 to 45 minutes. Records were kept of the order and time of each man's inoculation. Immediately after receiving an inoculation, each man was masked and returned to his room without having close contact with any other volunteer.

Post-Inoculation Studies. The clinical, laboratory and roentgenographic examinations were continued and intensified after inoculation. The temperature (oral), pulse and respiratory rates were recorded at 4-hourly intervals during the day, and at night when indicated. The development of symptoms and signs of respiratory disease were carefully noted by daily visits. Roentgenograms of the chest (PA view only), were taken routinely twice weekly. When indicated, the roentgenographic examinations were repeated at more frequent inter-

TABLE 2

Inoculation Procedure in Second Experiment

GROUP	TYPE OF INOCULUM	METHOD OF INOCULATION		
		First	Second	Third
A	Filtered	Power spray	Hand spray Medicine dropper	Hand spray
B	Autoclaved	Hand spray	Power spray	Hand spray Medicine dropper
C	Untreated	Hand spray Medicine dropper	Hand spray	Power spray

vals and included anterior-oblique views of the chest. Sinus films were repeated as indicated. Studies of the blood, including leukocyte and differential counts and determination of the erythrocyte sedimentation rate, were done twice weekly (*vide supra*). A sample of venous blood for serological study was withdrawn at weekly intervals. Bacteriological study of pharyngeal and nasal flora was continued once weekly.

Treatment. The treatment of patients was symptomatic; codeine sulphate, ½ to 1 grain, with or without phenobarbital, ½ to 1 grain, was given for symptoms of headache and cough. Antitussive agents, such as elixir terpine hydrate, with or without codeine sulphate, or Brown mixture were given freely for cough. Morphine sulphate, gr. $\frac{1}{8}$ to $\frac{1}{6}$,

was employed rarely for pain or restlessness. Barbiturates were used frequently as sedatives. Antipyretic drugs such as acetylsalicylic acid, phenacetine and phenetidin were not used. Oxygen was administered by means of a tent when indicated. Sulfadiazine was given to three patients because of complications.

B. THIRD EXPERIMENT

The third experiment was a repetition of the second experiment. Certain modifications in the experimental procedures were made, however, which included 1) enlargement of the volunteer group to 42 men, 2) changes in the method of segregation of the volunteers within the hotel, 3) the administration of inoculum out-of-doors and on separate days, and 4) the use of passage material obtained from patients in the previous experiment.

Subjects. The 42 "new" volunteers who comprised the group of subjects for this experiment varied in age from 18 to 37 years. All of the men were shown to be in good health.

Isolation Procedures. Eighteen men were housed on the third floor and 24 on the second floor. The latter were separated geographically into two groups of 12 men each by temporary partitions in the hallways. The quarantine restrictions were more rigid than those in the previous experiment. Transoms entering on the common hallways were kept closed constantly and doors were opened only to permit entrance of the professional and attending staffs. The isolation techniques were identical to those employed in the second experiment.

Pre-Inoculation Studies. The "control" period of isolation in this experiment varied between three and four weeks. During this time complete clinical, laboratory and roentgenographic studies were performed on each of the volunteers according to the program outlined above for the second experiment.

Inoculum. The inoculum consisted of pooled throat washings and sputum collected from six cases of primary atypical pneumonia occurring in the previous experiment. The donors all developed characteristic features of this disease including a rise in titer of cold hemagglu-

tinins to Group O human red cells (4c, 8). Throat washings in buffered Ringer's solution, as well as sputa, were collected daily from each donor during the period of acute febrile illness (Table 3). After culture, the specimens were stored in Pyrex test tubes at −70°C.

The procedures employed in preparation of the inoculum were the same as those in the previous experiment. Aerobic and anaerobic cultures of the filtered and autoclaved material were sterile. Culture of the untreated material showed the following organisms: alpha, alpha prime and gamma streptococci, *Staphylococcus aureus* (coagulase positive), diphtheroids, gram-negative cocci (not further identified) and one strain of streptococcus MG. Tubercle bacilli were not isolated.

Inoculation Procedure. Group A, consisting of 18 men, was given inoculations of autoclaved material. Four days later, a second group of 12 men (group B), was inoculated with filtered material. The final group of volunteers (group C), received untreated inoculum after a further interval of eight days.

The inoculations were given out-of-doors in order to minimize the opportunities for cross infection. Three areas within a vacant lot adjacent to the hotel were utilized for the separate inoculations. The volunteers were masked and escorted to an inoculation tent by a monitor. They left the hotel by one entrance and returned by another, and at no time was there close contact between any two volunteers. The professional personnel were gowned and masked during the entire inoculation procedure. Gowns and masks were changed between the inoculation of each individual.

Each volunteer received a total inoculum of 10 ml. administered in three equally divided doses throughout the morning and afternoon of a single day. As in the previous experiment, the material was sprayed into the nose and throat in synchronization with deep inspiration by means of an atomizer and vaporizer. Unlike the previous experiment, however, a tank of nitrogen was used as a source of pressure to vaporize the material instead of the motor-driven air pump, and none of the material was instilled into the respiratory tract with a medicine dropper. The inoculations were given by two teams and comparable techniques were maintained throughout. The separate inoculations given each volunteer consumed approximately 20 minutes. The

TABLE 3

Clinical and Laboratory Findings in Cases of Experimentally Induced Primary Atypical Pneumonia Selected as Donors of Specimens, Third Experiment

SPECIMEN					SERA		
Patients	Lobes involved	Type*	Day of disease	Cultural results †	Day of disease obtained§	Cold hemagglutinin titer	Streptococcus MG agglutinin titer
H. R.	R. U.	T. W.	1–6	*H. influenzae, H. hemolyticus,*	−4	<8	<8
	R. M.			streptococcus MG	3	<8	<8
	R. L.				10	<8	<8
	L. L.	Sp.	2, 4–6	Pneumococcus, type 17; strep-	17	16	<8
				tococcus MG	24	<8	<8
					52	8	<8
R. Y.	R. L.	T. W.	2, 3	Streptococcus MG	−3	<8	<8
	L. L.				4	<8	<8
		Sp.	3	— ‡	11	512	<8
					18	128	<8
					55	16	<8
R. T.	R. U.	T. W.	1–5	α Streptococcus, *H. hemolyticus*	−1	<8	<8
	R. M.			*Staphylococcus aureus*, hemo-	6	<8	<8
				lytic			
	R. L.				13	256	16
	L. L.	Sp.	2–5	α Streptococcus	20	256	16
					51	Hemolysis	32
H. D.	R. L.	T. W.	1–5	α and γ streptococci, *H. in-*	−4	<8	<8
				fluenzae			
	R. M.			*H. hemolyticus*, streptococcus	3	<8	<8
				MG			
	R. U.				10	512	16
	L. U.	Sp.	2–5	α, α′, γ streptococci, *H. in-*	17	256	32
				fluenzae			
	L. L.			*H. hemolyticus*, Pneumococcus,	24	64	32
				type 11, streptococcus MG	56	<8	16
H. B.	R. L.	T. W.	1–3	α streptococcus, *H. influenzae*	−4	<8	<8
	L. L.			*H. hemolyticus*, streptococcus	3	<8	<8
				MG	10	<8	<8
		Sp.	2, 3	*H. influenzae, H. hemolyticus*	17	32	<8
				Pneumococcus, type 11,	20	16	<8
				streptococcus MG	58	<8	<8
L. M.	R. L.	T. W.	1–6	*H. influenzae, H. hemolyticus,*	−4	16	<8
	L. L.			streptococcus MG	3	16	<8
		Sp.	4–6	α streptococcus, *H. influenzae*	10	2048	<8
				H. hemolyticus, streptococcus	17	1024	<8
				MG	24	512	8
					55	128	<8

* Sp. = sputum; T. W. = throat washings.

† Results of total organisms isolated in specimens of sputa or throat washings.

‡ Not cultured.

§ Numbers preceded by minus sign indicate days prior to inoculation.

equipment was sterilized during any session and between each set of inoculations by methods described in the previous experiment. Immediately after receiving an inoculation, each man was masked and returned to his room under escort.

Post-Inoculation Studies. The subjects were followed after inoculation in a manner identical with that described above. The experiment was terminated approximately four weeks after the last inoculation.

Treatment. The treatment of patients in this experiment was symptomatic. Sedatives and antitussive agents were employed freely. Oxygen therapy was given to one patient. Sulfonamide medication was not given. Antipyretic drugs were not used.

SUMMARY

A description is given of the methods employed in experimental attempts to transmit primary atypical pneumonia to human volunteers. Two experiments were performed, using groups of conscientious objectors as subjects, who were kept under quarantine restrictions for a period of approximately two months. The volunteers were inoculated into the respiratory tract with pooled sputa and throat washings obtained from cases of primary atypical pneumonia. The inocula in both experiments consisted of autoclaved, filtered and untreated material. The volunteers were observed closely before and after inoculation by clinical, laboratory and roentgenographic examinations.

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THE TRANSMISSION OF PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS*

II. Results of Inoculation

COMMISSION ON ACUTE RESPIRATORY DISEASES**
Fort Bragg, North Carolina

* This investigation was supported in part through the Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of The Surgeon General, United States Army, and by grants from the Commonwealth Fund, the W. K. Kellogg Foundation, the John and Mary R. Markle Foundation, and the International Health Division of the Rockefeller Foundation to the Board for the Investigation and Control of Influenza and Other Epidemic Diseases for the Commission on Acute Respiratory Diseases.

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In the preceding paper (1a) methods were described by which attempts were made to transmit primary atypical pneumonia to human volunteers. The present paper is concerned with an account of the results obtained in the second and third experiments of the series. A detailed analysis of the clinical and laboratory features observed in patients with experimentally induced pneumonia and minor respiratory illness is given in succeeding papers (1b, c).

A. SECOND EXPERIMENT

Atypical Pneumonia. In Table 1 are summarized the number and character of illnesses resulting from the three types of inoculum. Four subjects receiving filtered inoculum (Group A) and three receiving untreated material (Group C) developed characteristic symptoms, signs and roentgenographic evidence of primary atypical pneumonia. In addition, despite what were believed to be adequate isolation precautions, 3 individuals who received autoclaved inoculum (Group B) also developed primary atypical pneumonia.

Onset of disease in the four patients in Group A (filtered) occurred on the 8th, 12th, 12th and 14th days, respectively, after inoculation. Onsets in the three patients in Group C (untreated) all occurred on the 8th post-inoculation day. The three patients in Group B who received autoclaved material developed symptoms of disease 8, 10 and 10 days, respectively after inoculation.

The symptoms and signs observed at onset did not differ materially among the patients in the three groups. The course of the disease, however, showed considerable variation. In general, examples of mild, moderate and severe forms of atypical pneumonia were seen in each of the groups receiving filtered and untreated inoculum. The three individuals who received autoclaved inoculum and subsequently developed atypical pneumonia, had mild illnesses.

Complications were observed in two patients. Case No. 44, developed mild atypical pneumonia which was complicated by the occurrence of sterile pleural effusion. Case No. 47, suffered from a relatively mild atypical pneumonia but on the 18th post-inoculation day developed symptoms and signs of encephalomyelitis.

Roentgenographic lesions were characteristic of those seen in pa-

tients with primary atypical pneumonia in military and civilian hospitals. The appearance and regression of the pneumonic lesions in patients receiving the three types of inocula bore a striking similarity to those seen in the naturally occurring disease.

Cold hemagglutinins for Group O human erythrocytes developed in the sera of 8 of the 10 cases of atypical pneumonia. Agglutinins for the indifferent streptococcus MG developed during convalescence in the sera of 2 cases in a maximum titer of 32 from a pre-inoculation titer of <8. No change in titer was detected in the other 8 cases.

TABLE 1

Classification of Illness in Human Volunteers Inoculated with Autoclaved, Filtered and Untreated Sputa and Throat Washings from Hospital Cases. Second experiment

GROUP	TYPE OF INOCULUM	NO. MEN	RESULTS		
			AP*	MRI†	No illness
A	Filtered	12	4	5	3
B	Autoclaved	12	3	1	8
C	Untreated	12	3	9	0
Total.....		36	10	15	11

* AP = primary atypical pneumonia.

† MRI = minor respiratory illness of undifferentiated type.

Cultures of the throat, sputa and throat washings yielded no indication of a direct etiologic relationship between any single organism or group of organisms and atypical pneumonia (1c).

Minor Respiratory Illness. In addition to the 10 cases of primary atypical pneumonia there were 15 cases of minor respiratory illness of undifferentiated type (Table 1). The symptoms, physical signs, course and severity of these minor respiratory illnesses were variable (Table 2). Onsets of disease occurred between 1 and 11 days following inoculation. Constitutional symptoms of mild degree were noted at some time during illness by 10 of the 15 patients. Symptoms referable to the nose, throat or chest were encountered more frequently than constitutional symptoms, being present in all of the patients. Cough of mild degree was observed by 14 patients, in half of whom the cough was productive of scanty amounts of mucoid or muco-purulent sputum. Abnormal physical signs in the respiratory tract were noted

TABLE 2

Summary of Clinical Data Obtained from Patients with Minor Respiratory Illness. Second experiment

CASE NO.	TYPE OF INOCULUM	ONSET	SYMPTOMS AT ANY TIME*											PHYSICAL SIGNS AT ANY TIME†										DAYS FEVER >99.6°	SEROLOGY MAX. TITER	
			Constitutional					Local							Nasal			Pharynx tonsils				RALES				
		Days after inoculation	Ch.	F.	H.	M.	An.	Nasal	S. T.	Ho.	C.	Sp.	Ch. P.	C.A.	Inj.	Obs.	Disch.	Inj.	Ex.	Lymph hyp.	Lymph nodes	Fine	Coarse		Cold agg.	Strep. MG
29	Filtered	11	0	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
33	Filtered	9	0	+	+	+	+	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
27	Filtered	10	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0	+	0	0	<8	8
35	Filtered	7	0	0	+	+	0	+	0	0	+	+	0	0	0	0	0	0	0	+	0	0	0	0	<8	<8
14	Filtered	4	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8‡	<8
13	Untreated	6	0	0	+	0	0	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	16§	8
28	Untreated	1	0	+	+	0	0	+	+	+	+	+	+	0	0	+	+	+	0	+	+	0	0	0	8‖	8
11	Untreated	4	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	0	0	0	+	0	0	0	<8	<8
15	Untreated	3	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
34	Untreated	1	0	+	+	+	+	+	+	0	+	0	0	0	0	0	0	0	+	+	0	0	0	1	8‡	<8
32	Untreated	1	0	0	0	0	0	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
31	Untreated	6	0	0	0	0	0	0	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
30	Untreated	2	+	0	+	+	0	0	+	+	+	+	0	0	0	0	0	0	+	+	0	0	0	1	<8	<8
9	Untreated	4	0	0	+	0	0	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	<8	<8
22	Autoclaved	7	0	+	+	0	0	+	+	+	+	0	+	0	0	0	0	0	0	+	0	0	0	1	<8	<8

* Symptoms—Constitutional: Ch. = chilliness; F. = feverishness; H. = headache; M. = malaise; An. = anorexia. Local: S.T. = sore throat; Ho. = hoarseness; C. = cough; Sp. = sputum; Ch.P. = chest pain.

† Physical signs—C.A. = clinical appraisal (0 = not ill); nasal: Inj. = injection; Obs. = obstruction; Disch. = discharge; Pharynx-tonsils: Inj. = injection; Ex. = exudate; Lymph. hyp. = lymphoid hyperplasia, pharynx.

‡ Pre-illness titer: 8.

§ Titer on 11th day after inoculation (5th day after onset); pre-illness titer: 8.

‖ Titer before inoculation.

in only seven patients despite the existence of localizing or constitutional symptoms occurring simultaneously. Exudative pharyngitis, developing within 48 hours of inoculation, was present in two patients, both of whom received the untreated inoculum. Although cough was noted by all but one of the patients, only one individual, Case No. 27, developed abnormal lung signs consisting of transitory fine rales. Fever of 99.6° or above was present in four patients.

Cold hemagglutinins were observed in the serum of one patient with minor respiratory illness. This individual, Case No. 13, showed a change in titer from <8 before inoculation to 16 on the 11th day after inoculation (fifth day after onset). Agglutinins for the streptococcus MG were not detected in any of the samples of serum.

Weekly cultures of the pharynx and nose of these patients yielded normal bacteriological flora in most instances. Pneumococci were isolated from 14 patients; the types found conformed to those generally considered to be "carrier types." Beta-hemolytic streptococcus was isolated from a total of four patients, in one individual, Case No. 28, before and not after inoculation; in the remaining three, Cases Nos. 33, 34, 35, after inoculation only. Each of the strains isolated from these three patients was Group A, no type. In one individual, Case No. 34, exudative pharyngitis was present.

No Illness. From Table 1 it will be seen that 11 patients experienced no illness. Three of this group were inoculated with filtered material and 8 with autoclaved inoculum. Data concerning a preceding history of respiratory disease and bearing upon the possible relationship of previous respiratory experience and susceptibility to atypical pneumonia yielded little information of value in these 11 individuals, except in one instance. One individual, Case No. 17, who received filtered inoculum had had atypical pneumonia 3 months prior to inoculation.

Illnesses in Professional Staff and Attendants. Primary atypical pneumonia developed in two individuals who were intimately exposed to the material given on the day of inoculation. Brief case reports follow:

A. E. F. participated actively in the laboratory in the preparation of all three types of inoculum. On the day of inoculation, 26 June 1944, he prepared and distributed for use all specimens in the inoculation rooms.

On 8 July, 12 days after the inoculations, while working in the laboratory, he experienced sudden onset of generalized malaise and chilliness. These symptoms increased in intensity forcing him to bed. No elevation of temperature was discovered. Later that day marked generalized aching and chilliness were observed.

The following day he observed slight sore throat and frontal headache in addition to the above-mentioned constitutional symptoms. His temperature rose from 99.5°F. that morning to 101°F. in the evening. No abnormal physical signs were detected. During the next three days, an irregular febrile illness ensued marked by dry cough, minimal symptoms from the nose and throat and headache. A roentgenogram on 11 July showed a soft area of density in the peripheral portion of the right middle lobe. A few fine inspiratory rales were detected over this area. The leukocyte count was 8050 and the differential showed polymorphonuclear cells, 63 per cent; lymphocytes, 37 per cent.

Symptoms of nasal obstruction and discharge occurred for a short period during convalescence, beginning approximately a week after cessation of the febrile period. A roentgenogram on 21 July showed a residual area of infiltration. Convalescence was protracted because of marked fatigability lasting for approximately three weeks.

T. W., an attendant, assisted in inoculating 12 of the volunteers with untreated inoculum. For approximately 5 hours on 26 July 1944 he was confined to one of the inoculating rooms. On 7 July, 11 days following this intimate exposure, he noted chilliness, feverishness, headache and dryness of the throat. The following day these symptoms were accompanied by a productive cough and by fever (101.6°F.). A soft infiltration in the left lower lobe was first visible by roentgenogram on 10 July. Physical signs were noted over the area of infiltration beginning on 11 July. The febrile course was of 6 days duration. The lungs cleared rapidly and there were no complications. The diagnosis was primary atypical pneumonia of mild severity.

Leukocyte and differential counts during this illness showed normal levels. Cold hemagglutinins were present in a titer of 128 in a sample of serum taken 14 days after the development of respiratory symptoms. A rise in titer of agglutinins for streptococcus MG was not detected.

Comment. It is apparent from the results of this experiment that primary atypical pneumonia, as well as minor respiratory illness, developed in human volunteers who were inoculated with secretions of the respiratory tract of patients with atypical pneumonia. It is also evident that two individuals who presumably were exposed to heavy concentrations of the inoculum, on or shortly before the day of inoculation, likewise developed primary atypical pneumonia.

Three cases of pneumonia and one of minor illness developed among those receiving autoclaved inoculum. This event was not anticipated and several possibilities were considered as likely explanations. (*1*) *Contamination of the inner surface of the air-pump.* This pump was used first to inoculate a group of volunteers with filtered inoculum and secondly to inoculate autoclaved material. Although the nasal attachments of the power spray were sterilized between inoculations, the inner surface of the air pump was not sterilized. It is possible that air-borne particles of the filtered material, trapped in the air pump, could have been liberated subsequently when the power spray was used with the autoclaved material. It is perhaps of some significance that, of the 12 men who received autoclaved inoculum, the 3 who developed atypical pneumonia were the first, second and fourth in order of inoculation with the power spray. The individual who developed minor illness was the eighth in order of inoculation. (*2*) *Air-borne infection at the time of inoculation.* If the assumption is made that air-borne infection did account for these illnesses it is logical to suppose that exposure occurred on the day of inoculation in the hallway outside of the inoculation rooms. During the process of waiting, which in some cases was unavoidably long, some of the men could have contracted infection as a result of inhalation of air-borne droplets or droplet nuclei. The doors leading from the inoculation rooms were opened frequently to permit entrance and exit of the subjects. Furthermore, the occurrence of atypical pneumonia not only in one attendant who assisted in the inoculation of untreated material, but also in one member of the professional staff who supervised the preparation and distribution of the inocula suggested air-borne infection. (*3*) *Cross infection after inoculation.* Despite the isolation precautions, cross infection after inoculation, through air-borne droplets or droplet nuclei, might have explained the occurrence of cases in the

"autoclaved" group. This possibility seemed unlikely in view of the absence of concentration of cases in any part of the hotel and also because of the time of development of disease in these subjects. One individual noted symptoms beginning 8 days, and two, 10 days after inoculation. The incubation period in these cases thus was consistent with that observed in other individuals who received filtered inoculum. (*4*) *Non-specific evocation of a latent agent.* The theoretical possibility existed that illnesses in the autoclaved group resulted from the stimulation of a latent agent by the inoculations. Although no evidence for this hypothesis was at hand, a situation analogous to herpes infection in man or infection with pneumonia viruses in mice (2, 3) may have existed. It was apparent that evidence bearing on this hypothesis could only be obtained by a repetition of the experiment using extreme precautions against air-borne infection or other sources of cross-infection. (*5*) *Errors in preparation and administration of the inocula.* Because of the extreme precautions taken in preparing and distributing the various types of inocula it seemed unlikely that any errors in technique had occurred. Furthermore, sterilization of the autoclaved material was considered to be adequate in view of the fact that aerobic and anaerobic cultures were sterile.

In summary, then, the results of this experiment indicated that primary atypical pneumonia had been transmitted and that the disease was transmitted by air-borne infection. No conclusion as to the nature of the etiological agent, however, could be drawn from this study. Accordingly, the experiment was repeated with modifications as follows: (*1*) The inoculations were given out-of-doors and on different days and a tank of nitrogen supplanted the motor-driven power-spray. (*2*) Additional precautions to prevent inadvertent air-borne cross-infection after inoculation were instituted by the erection of partitions in the hallways between groups of patients. In addition, transoms of each room were kept closed constantly, and the doors were opened only to permit entrance of the staff. (*3*) The volunteer group was enlarged to increase the possibility of demonstrating the existence of a latent agent. (*4*) Inocula obtained from patients with pneumonia occurring in the previous experiments were utilized in order to provide opportunity for passage of the agent.

B. THIRD EXPERIMENT

Atypical Pneumonia. In this experiment there were six individuals who developed illnesses characteristic of primary atypical pneumonia (Table 3). Three of these patients received filtered inoculum (Group B) and three received untreated material (Group C). There were no cases of atypical pneumonia among individuals who received autoclaved inoculum (Group A).

The symptoms, physical signs and clinical course of disease observed in these six patients bore a striking similarity to those observed, not only in the donors of the inoculum (passage material from experi-

TABLE 3

Classification of Illness in Human Volunteers Inoculated with Autoclaved, Filtered and Untreated Sputa and Throat Washings from Patients with Experimental Atypical Pneumonia. Third experiment

GROUP	TYPE OF INOCULUM	NO. MEN	RESULTS		
			AP*	MRI†	No illness
A	Autoclaved	18	0	1	17
B	Filtered	12	3	5	4
C	Untreated	12	3	5	4
Total.....		42	6	11	25

* AP = primary atypical pneumonia.
† MRI = minor respiratory illness of undifferentiated type.

mental illnesses), but also in the hospitalized donors of the original inoculum. Although the illnesses were somewhat milder in patients in the "filtered" group than in the "untreated" group there were no other essential differences in the symptoms and signs at onset and during the course of the disease in the two groups.

Onset of disease in three patients in Group B (filtered) occurred on the 12th, 13th and 14th days, respectively, after inoculation. Onsets occurred earlier in the three patients who became ill after receiving untreated material (Group C); two developed on the 7th day, and one on the 8th day after inoculation.

The appearance and regression of the roentgenographic lesions in the patients from both "filtered" and "untreated" groups were similar

to those seen in the naturally acquired, as well as the experimentally induced disease. In three individuals single lobes were involved, in two patients bilateral lesions were observed, and in one individual all five lobes were involved in association with a critical illness.

Cold hemagglutinins developed in the sera of five of the six cases of pneumonia. In Group B (filtered) maximum titers of 128, 1024 and 1024 were obtained on the 27th, 27th and 20th days, respectively, after inoculation. Two patients in Group C (untreated) developed maximum titers of 512 and 8192 on the 21st and 26th days, respectively, following inoculation. A third patient did not develop cold agglutinins at any time.

A rise in titer of agglutinins for streptococcus MG was not demonstrated in the sera of any of the six patients with pneumonia.

As noted in the previous experiment cultures of the throat, sputa and throat washings yielded no indication of a direct etiologic relationship between any bacteria and atypical pneumonia.

Minor Respiratory Illness. There were eleven individuals who developed minor illnesses following inoculation (Tables 3 and 4). In 10 of these patients onset of illness occurred between 5 and 15 days after inoculation; in one individual, onset was somewhat later, the 19th day. This person was the only member of the "autoclaved" group who became ill. As far as was known, he was the only volunteer who "broke" isolation; on at least one occasion he descended a fire escape to converse with a member of Group C, Case No. 138, (Table 4), who subsequently developed "suspected atypical pneumonia" (vide infra).

As was noted in the previous experiment, the clinical features of these minor respiratory illnesses were variable (Table 4). Seven illnesses were extremely mild with minimal symptoms and signs. Four illnesses were more severe, characterized by fever and moderate symptoms and physical signs of respiratory disease. These cases, Nos. 124, 134, 131 and 138, were diagnosed as "suspected atypical pneumonia" because the clinical course, including the presence of rales, was characteristic of atypical pneumonia, although no roentgenographic evidence of pulmonary infiltration was found (4).

Cold hemagglutinins were observed in the sera of nine patients.

TABLE 4

Summary of Clinical Data Obtained from Patients with Minor Respiratory Illness. Second experiment

CASE NO.	TYPE OF INOCULUM	ONSET	SYMPTOMS AT ANY TIME*											PHYSICAL SIGNS AT ANY TIME†												DAYS FEVER > 99.6°	SEROLOGY MAX. TITER	
			Constitutional					Local						C.A.‡	Nasal			Pharynx tonsils		Lymph hyp.	Lymph nodes	Rales				Cold agglu.	Strep. MG
		Days after inoculation	Ch.	F.	H.	M.	An.	Nasal	S.T.	Ho.	C.	Sp.	Ch. P.		Inj.	Obs.	Disch.	Inj.	Ex.			Fine	Coarse				
110	Filtered	15	0	0	0	0	0	+	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	64	<8
124	Filtered	9	+	+	+	+	+	+	+	+	+	+	+	1	0	+	0	+	0	+	+	+	+	5	8	<8
113	Filtered	10	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	<8	<8
130	Filtered	12	+	+	+	+	+	0	+	0	+	0	+	1	0	0	0	0	0	+	+	0	+	+	2	16	<8
134	Filtered	12	+	+	+	+	0	0	+	0	+	+	0	1	0	0	0	0	0	+	0	+	+	2	64	8
127	Untreated	6	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	<8
129	Untreated	7	0	0	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	0	0	+	0	16	32
131	Untreated	8	+	+	+	+	+	+	+	0	+	+	+	2	0	0	0	0	0	0	0	+	+	2	256	<8
103	Untreated	6	0	0	0	+	+	0	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	128	<8
138	Untreated	5	0	+	+	+	+	+	+	+	+	+	+	2	0	0	0	0	0	0	0	+	+	2	16	8
102	Autoclaved	19	0	0	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	16	<8

* Symptoms—For explanation of abbreviations see legend, table 2.

† Physical Signs—For explanation of abbreviations see legend, table 2.

‡ C.A. = clinical appraisal (0 = not ill; 1 = mildly ill; 2 = moderately ill).

Four individuals showed titers of 64 or greater and five had titers of less than 64, although a demonstrable rise was present in each case. Agglutinins for streptococcus MG in a titer of 32 were noted in the serum of one patient, Case No. 129.

Cultures of the throat and nose at weekly intervals revealed normal bacteriological flora. Pneumococci, generally of the higher types, were isolated from 7 patients. Beta-hemolytic streptococci were recovered from 4 individuals, in two when first observed but never subsequently; in one, just prior to discharge and in one both before and at the end of the experiment. In none of these patients was exudative pharyngitis or tonsillitis present.

No Illness. In addition to the 17 persons in Group A (autoclaved) who suffered no illness, there were four volunteers in Group B (filtered) and four in Group C (untreated) who experienced no respiratory symptoms or signs (Table 3). None gave a history of atypical pneumonia.

Illness in Professional Staff. One individual who assisted in the inoculations developed mild atypical pneumonia.

I. G. participated in the preparation of the inoculum on 22 August 1944. On 28 August and 5 September he administered filtered and untreated inoculum, respectively, to a group of volunteers. He was particularly careful to wear two cotton masks as well as a plastic head mask during the entire inoculation procedure on each day.

On 17 September, 20 days after administering filtered inoculum and 12 days after giving untreated material, he observed gradual onset of anorexia and malaise. The following day headache, chilliness, feverishness and dry cough were noted. Temperature readings varied between 100° and 103.4°F. Roentgenogram of the chest was negative.

During the next 7 days an irregular febrile course was observed, characterized by cough, at first dry and later becoming productive, substernal discomfort, minimal nasal symptoms and moderate constitutional symptoms. Auscultatory signs and roentgenographic evidence of pneumonic infiltration developed in the right lower lobe on the 5th day of disease (22 September). Convalescence was uneventful but was marked by a prolonged period of fatigability.

DISCUSSION

Etiological Considerations. The results of experiments two and three provide evidence for the transmission of primary atypical pneumonia through two successive groups of well persons. Atypical pneumonia was induced by bacteria-free filtrates of sputa and throat washings as well as by the same pool of material before filtration. In the third experiment, the inoculation of autoclaved material resulted in no illnesses which could be ascribed to this procedure. It thus appeared probable that the cases following inoculation of autoclaved material in the prior experiment had resulted from inadvertent infection. Furthermore, the failure of autoclaved material to induce respiratory illness was evidence against the presence and non-specific stimulation of a latent agent. Thus, the etiologic agent appeared to be filtrable and was presumably viral in nature.

The possibility that more than one filtrable agent was operative in these experiments should be considered. The development of minor respiratory illnesses of variable clinical picture in those receiving the filtrate was suggestive of this. Furthermore, the use of pooled material from several donors, theoretically at least, favored this hypothesis. The studies reported here do not provide sufficient data by which this hypothesis can be substantiated; neither do they disprove it.

The concept that bacteria may act in conjunction with a filtrable agent in the production of atypical pneumonia should also be considered. The failure to detect significant changes in bacterial flora by weekly throat cultures does not exclude this possibility, although it is reasonable to assume that had the changes been striking they would have been detected by the methods employed.

Epidemiological Considerations. The incubation period of experimental atypical pneumonia was found to be essentially the same in the two experiments. Persons receiving untreated material developed symptoms of disease approximately one week after inoculation. The onset of illness in those inoculated with filtrate was approximately two weeks after inoculation. It is probable that this discrepancy arose as a result of a decrease in dosage occasioned by the filtration process. Thus it is evident that the incubation period of the experimental disease may be as short as one week or as long as two weeks. Whether

these observations reflect the chain of events which occurs in the naturally-acquired infection cannot be stated. The significance of dosage, the virulence of the virus and the immune status of the individual have yet to be determined in the naturally-acquired disease.

The experimental data provide information regarding the mode of transmission of primary atypical pneumonia. It is generally assumed that infection may occur through droplets, droplet nuclei or by contact with an infected person. Other means such as contact with freshly soiled linen, handkerchiefs, etc., have also been thought to play a rôle. Evidence in support of air-borne infection was obtained in the occurrence of pneumonia in one attendant and two members of the staff who were highly exposed to the infectious agent. Moreover, air-borne infection at the time of inoculation was the probable modality in the three cases in the "autoclaved" group. No evidence was obtained regarding soiled linen, food, water, milk, eating utensils or insect vectors as factors in transmission of the disease.

The occurrence of minor respiratory illnesses in approximately half of the subjects inoculated with filtered and untreated material suggested a relationship of this group of diseases to primary atypical pneumonia. The clinical similarity in some cases was striking, particularly in those who had moderately severe illnesses and exhibited respiratory symptoms, rales and cold hemagglutinins. Roentgenographic evidence of pulmonary infiltration, however, was lacking. These illnesses not infrequently resembled those observed on the respiratory wards of Army hospitals. The observations reported there thus provide experimental evidence in favor of the existence of a *non-pneumonic* form of primary atypical pneumonia. The existence of this type of disease has been suggested by Gallagher (5a, b) and by Reimann and associates (6a, b). Epidemiological evidence has supported this hypothesis to a limited degree (4). Proof of this relationship has not yet been obtained, however, and must await further etiological studies.

Limited data were obtained bearing on the question of human susceptibility to infection. Excluding the controls who were inoculated with autoclaved material, three-fourths of the patients developed some type of respiratory disease; one-fourth had atypical pneumonia and one-half minor respiratory illness.

SUMMARY

Two experiments are described wherein primary atypical pneumonia was transmitted to human volunteers. The inoculum consisted of a pool of sputa and throat washings, obtained from individuals with characteristic illness, which was sprayed into the respiratory tract of presumably susceptible subjects. In one experiment the inoculum was provided by seven hospitalized soldiers; in the other, it was obtained from six experimentally induced cases of atypical pneumonia. Filtered, untreated and autoclaved inoculum was employed in each experiment.

Respiratory illnesses having all of the characteristic features of primary atypical pneumonia were observed in individuals in both experiments who received untreated or filtered inoculum. The infection was thus carried through two successive groups of well persons. When precautions during inoculation were rigid, no cases of pneumonia developed in any of the individuals who received autoclaved inoculum.

The results of these experiments indicate that bacteria-free filtrates, presumably containing a virus, can induce primary atypical pneumonia in man. The incubation period of the experimental disease was approximately one week in subjects who received untreated inoculum and approximately two weeks in those inoculated with the filtrate.

The occurrence of minor respiratory illness in subjects receiving both types of inoculum suggested a relationship of this group of diseases to atypical pneumonia.

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THE TRANSMISSION OF PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS*

III. Clinical Features

COMMISSION ON ACUTE RESPIRATORY DISEASES**
Fort Bragg, North Carolina

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In preceding papers there were described the techniques employed and the results obtained in experiments two and three on the transmission of primary atypical pneumonia to human volunteers (1a, b). This paper presents a detailed analysis of the clinical data collected from 16 cases of atypical pneumonia and 26 cases of minor respiratory illness occurring in both experiments. A summary of the laboratory findings in these same cases is given in a succeeding paper (1c).

Onset and Incubation Period. Because each of the experimental subjects was observed at least once daily after inoculation a unique opportunity was afforded in this study to observe the development of symptoms and signs and thereby to determine as accurately as possible the incubation period of the experimental disease.

Upon analysis of the data it soon became apparent, however, that the clinical onset in these cases was as difficult to define as in naturally-acquired infections. There were a few cases in which the onset was easily determined because of the intensity of certain developing symptoms or the appearance of abnormal objective signs, such as fever. In other cases, the mild symptoms and the lack of objective signs made determination of onset difficult. Arbitrary definition of clinical onset in atypical pneumonia thus became necessary, and it was defined as "the day of development of *persistent* symptoms or signs which resulted in atypical pneumonia." The clinical onset of minor respiratory illness was defined as "the day of development of any recognizable symptom or sign which was a definite departure from the normal state for that individual but which did not lead to atypical pneumonia." The frequency of transient local symptoms without recognizable signs of illness occurring among the cases of atypical pneumonia, and the short duration of some cases of minor respiratory illness justified these arbitrary and distinctive definitions.

The incubation period of atypical pneumonia thus delineated, varied from 7 to 14 days, whereas the incubation period of minor respiratory illness varied from one to 19 days (Table 1). Seven cases of pneumonia occurred among individuals receiving filtrate, six of whom developed symptoms of pneumonia between 12 and 14 days after inoculation. The onset of illness in those inoculated with untreated

material was approximately one week earlier. Similar, although not identical, differences were noted in patients with minor illness. Persons receiving untreated material developed symptoms within 8 days after inoculation; the majority of patients receiving filtered inoculum developed symptoms during the second week.

TABLE 1

Incubation Period in Experimentally Induced Primary Atypical Pneumonia and Minor Respiratory Illness

DAYS AFTER INOCULATION	NUMBER OF CASES		
	Atypical pneumonia		Minor respiratory illness
	Onset of definite illness	Onset of first abnormal symptom or sign	Onset of first abnormal symptom or sign
1		1	3
2		1	1
3			1
4		2	3
5		3	1
6		1	4
7	2	2	3
8	6	2	1
9			2
10	2	1	2
11		1	1
12	3	1	2
13	1		
14	2	1	
15			1
19			1
Total cases.....	16	16	26

The character of onset varied considerably among the 16 cases of atypical pneumonia. In 5 cases the simultaneous occurrence of constitutional and local symptoms, together with fever on the same day, marked the sudden onset of illness. In the remaining 11 cases preceding local or constitutional symptoms, transient in character and developing from the 1st to 11th day after inoculation, signified the gradual onset of clinical illness. The gradual character of onset found in the majority of these patients thus conformed to the findings

usually observed in the naturally-acquired infection (2, 3). As noted above, symptoms preceding the "onset" of atypical pneumonia were noted in 11 patients. Dry or sore throat was the first local symptom noted by 7 patients; in three of the latter, hoarseness was also present. In three additional patients nasal symptoms were the first indication of departure from health. In the remaining individual, cough was the initial preceding symptom. Headache was noted by three of the 11 patients in association with local symptoms The presence of these symptoms transient in character and often preceding the development

TABLE 2

Total Frequency of Symptoms in 16 Cases of Experimentally Induced Primary Atypical Pneumonia and 26 Cases of Minor Respiratory Illness

SYMPTOMS	ATYPICAL PNEUMONIA		MINOR RESPIRATORY ILLNESS	
	No. cases	Per cent	No. cases	Per cent
Constitutional:				
Chilliness	13	81	4	15
Feverishness	14	87	9	34
Headache	15	93	17	65
Malaise	12	75	12	46
Anorexia	16	100	8	30
Local:				
Nasal (sneezing, obstruction, discharge)	12	75	16	61
Sore throat	11	68	15	57
Hoarseness	12	75	9	34
Cough	16	100	21	80
Sputum	14	87	11	42
Chest pain (substernal)	14	87	9	34

of clinical disease by several days, suggested either the existence of a prodromal period of infection, or the occurrence of minor respiratory illness in addition to atypical pneumonia in the same patient.

Symptomatology. The total frequency of symptoms observed in the cases of atypical pneumonia and minor respiratory illness is given in Table 2. Constitutional symptoms of chilliness, feverishness, headache, malaise and anorexia each occurred in three-quarters of the patients with pneumonia. Anorexia was noted at some time by all of these patients. Headache, usually frontal in type but sometimes generalized, and of variable intensity was present in 15 patients.

Feverishness and chilliness were common; definite rigor was not experienced. Malaise, a term used to include generalized aching or pain in muscles or joints, was observed by 12 patients. Vomiting was not encountered. In patients with minor respiratory illness headache was the most frequently observed symptom and one individual experienced a true rigor. Other constitutional symptoms were present in lessened frequency indicative of the generally mild character of illnesses in this group.

The manner of development of certain of these constitutional symptoms in relation to onset has been studied in the two groups of cases. Figure 1 depicts the frequencies with which individual symptoms occurred by days throughout the first 2 weeks from onset. It will be noted that headache and feverishness developed early in patients with pneumonia and were present for 6 days in approximately half of the cases. The maximum prevalence of chilliness occurred on the 2d day. Malaise was present in 56 per cent of the cases as early as the second day and thereafter was observed in decreasing frequency. Anorexia (curve not shown on chart) was noted in increasing frequency during the first three days, reaching a maximum of 87 per cent of the cases at this period and thereafter showing a rapid decline. The frequency curves for minor respiratory illness (Figure 1) show the lessened incidence of specific symptoms in comparison to those observed in atypical pneumonia and slight differences in pattern. These were most pronounced in the case of chilliness and the least evident in the case of malaise where the curves were roughly parallel.

Local symptoms were present at some time during illness in the majority of patients with atypical pneumonia. In some patients with minor illness they comprised the sole characteristic features of disease. Nasal symptoms and sore throat occurred with approximately equal frequency in the two groups of cases (Table 2, Figure 2). Hoarseness in atypical pneumonia was noted with increasing frequency up to a maximum of 70 per cent on the 3d day and was of mild severity. In the minor illness group, hoarseness occurred with gradually increasing frequency until the 5th day. Cough, usually dry at first and later becoming constant and paroxysmal in character, was one of the most conspicuous features of atypical pneumonia and also minor illness. Sixty per cent of cases of pneumonia had cough present on the first day

of illness, and all patients experienced this symptom by the 4th day. In patients with minor illness cough was most frequently observed also on the 4th day.

Sputum, usually mucoid in the early stages and later muco-purulent or purulent, was noted by 14 of the 16 patients with atypical pneumonia and by 11 of 26 individuals with minor illness (Table 2). Only two patients who were severely ill with pneumonia manifested blood-

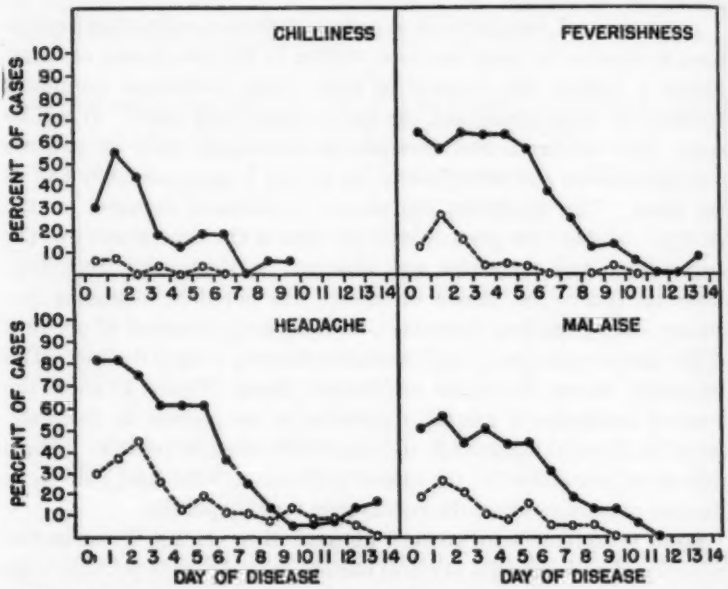
CHILLINESS
FEVERISHNESS
HEADACHE
MALAISE
PERCENT OF CASES
DAY OF DISEASE
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
10 20 30 40 50 60 70 80 90 100

FIG. 1. Daily frequency of various constitutional symptoms in 16 cases of experimentally induced primary atypical pneumonia (solid lines) and in 26 cases of minor respiratory illness (dotted lines).

streaked sputum. Grossly bloody or "rusty" sputum was not observed. The character of sputum in patients with minor illness resembled that seen in cases of pneumonia. The character of the "sputum frequency curve" in the experimental cases of atypical pneumonia (Figure 2) supports the clinical impression of the relatively late development of sputum in the natural disease. Fourteen patients with pneumonia experienced chest pain which was variously described as a sense of discomfort, pressure or burning in the substernal

region. The prevalence of this symptom in most cases was similar to that of cough with which it seemed to be intimately associated. In

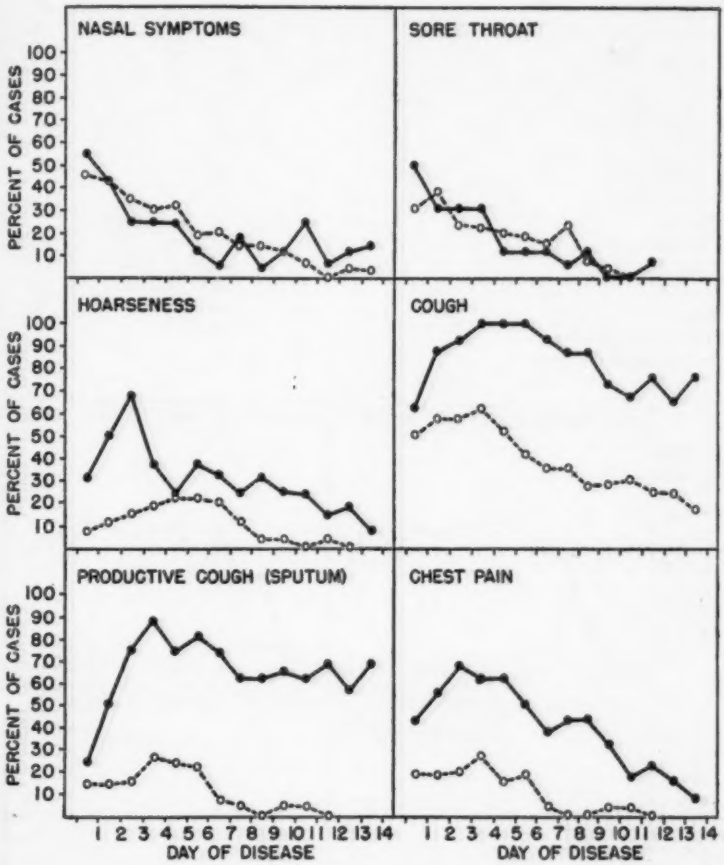

FIG. 2. Daily frequency of various local symptoms in 16 cases of experimentally induced primary atypical pneumonia (solid lines) and in 26 cases of minor respiratory illness (dotted lines).

only one case was pleuritic pain observed. Chest pain was present in one-third of patients with minor illness.

TABLE 3

Total Frequency of Prominent Physical Signs in 16 Cases of Experimentally Induced Primary Atypical Pneumonia and 26 Cases of Minor Respiratory Illness

PHYSICAL SIGNS	ATYPICAL PNEUMONIA		MINOR RESPIRATORY ILLNESS	
	No. cases	Per cent	No. cases	Per cent
Pulmonary:				
Fine rales	15	94	5	19
Coarse rales	15	94	5	19
Impaired expansion	2	12	0	0
Dullness	10	62	0	0
Diminished fremitus	5	31	0	0
Altered breath sounds	7	43	0	0
Nasal:				
Obstruction	10	62	4	15
Discharge	6	37	4	15
Pharynx:				
Lymphoid hyperplasia	6	37	8	30
Exudate	2	12	3	11
Cyanosis	5	31	0	0
Herpes	0	0	0	0
Cervical adenopathy	0	0	4	15
Central nervous system	2	12	0	0

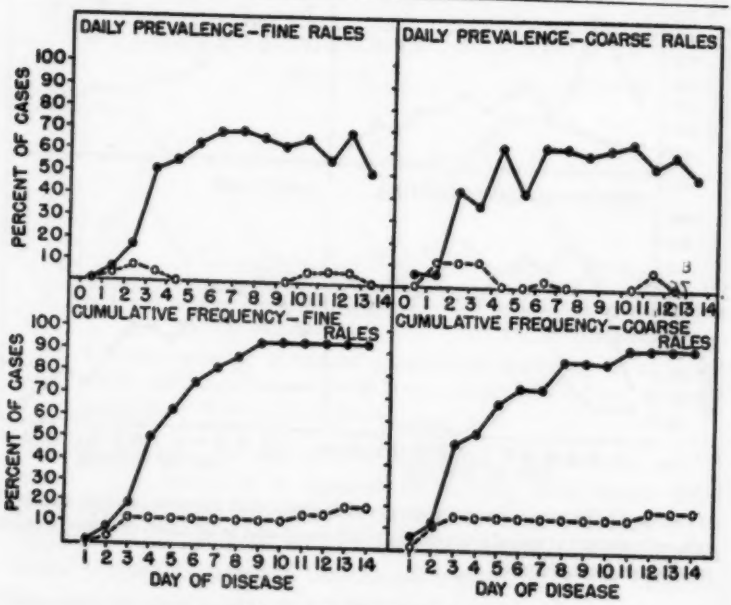

FIG. 3. PHYSICAL SIGNS

Daily prevalence and cumulative frequency of fine and coarse rales in 16 cases of experimentally induced primary atypical pneumonia (solid lines) and 26 cases of minor respiratory illness (dotted lines).

Physical Signs. A summary of the total frequency of occurrence of prominent physical signs is given in Table 3. Fine and coarse rales were each present in 15 of the 16 patients with pneumonia. One patient showed no rales at any time. In Figure 3 the daily prevalence and cumulative frequency of fine and coarse rales are compared. It is apparent that in the first three days of disease less than 20 per cent showed fine rales and that by the fourth day these rales were elicited in half of the patients. The greatest increment of new cases showing this sign occurred from the third to the fourth day. By the ninth day 15 of the 16 patients had developed fine rales.

The pattern of development of coarse rales was roughly parallel to that of fine rales with certain exceptions. Half of the cases showed coarse rales by the third day, and not until the 11th day did the maximum frequency occur.

Other lung signs exclusive of rales were noted in approximately two-thirds of the patients. These consisted of impaired expansion (2 cases), slight percussion dullness (10 cases), changes in tactile or vocal fremitus (5 cases), and alteration in character of the breath sounds in 7 cases. Signs of pleural fluid were detected in one patient. Friction rub was not observed.

The presence of pulmonary signs (fine and coarse rales) was detected in approximately five patients with minor respiratory illness. Other lung signs were not observed in this group.

Nasal obstruction was noted in 10 patients with pneumonia. In six there was accompanying nasal discharge. In patients with minor illness nasal symptoms were infrequent.

Approximately one-third of patients in both diagnostic categories showed abnormal prominence of pharyngeal lymphoid follicles. Pharyngeal or tonsillar exudate of minimal degree was present in 2 cases of pneumonia and in 3 who had minor illness. Cervical adenopathy was not observed in the pneumonia group, but was noted in four individuals with minor illness.

Cyanosis of mild degree was present in 5 patients; three of these individuals were severely ill and had extensive pneumonic infiltration. Herpes was not observed in any of the patients.

Signs of involvement of the central nervous system were noted in two patients. One of these developed signs of encephalomyelitis with partial loss of bladder and rectal function and motor and sensory

impairment of the lower limbs. In the other patient transient areflexia was observed in the lower limbs.

Roentgenography. The earliest roentgenographic sign in 10 patients was peribronchial infiltration alone without concomitant hilar involvement. Most commonly seen at the lung bases and best visualized by anterior-oblique films, this type of infiltration appeared either linear as an exaggeration of the basilar truncal markings or as patches of soft irregular density (Figure 4). Such lesions frequently were located in the most peripheral and dependent portions of the lungs, the sulci, and connection with the normal hilar shadow did not appear to be con-

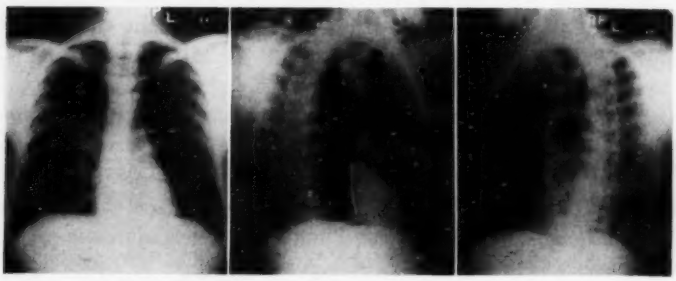

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FIG. 4, CASE NO. 42

Roentgenogram of patient with mild atypical pneumonia. Anterior-oblique films show early infiltration at both bases; posterior-anterior film is clear.

tinuous. The earliest sign in the remaining 6 cases was unilateral or bilateral perihilar infiltration with or without parenchymal involvement of one or more lobes (Figure 5).

The *appearance* of the pneumonic lesions in these 16 patients resembled the radiographic picture observed in the natural disease. Soft, patchy densities of irregular size and shape were commonly found (Figure 6) although in a few patients the lesions were of uniform ground glass appearance with lung markings usually visible throughout the area. Occasionally, the densities were small, discrete and nodular in the early stages, later enlarging in size and becoming patchy and confluent. The resemblance of this type of infiltration to miliary tuberculosis was striking. In only a few patients, those with marked perihilar involvement, were the lesions as dense as those seen in pneumococcal pneumonia.

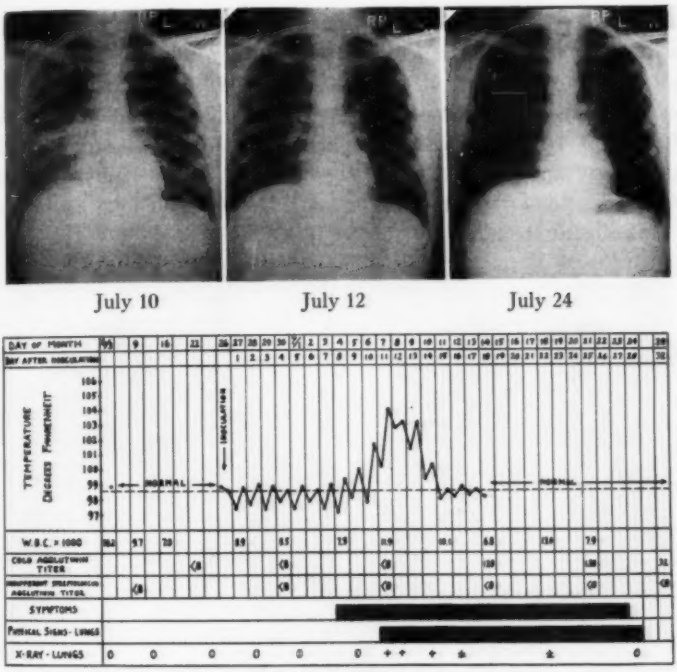


FIG. 5, CASE NO. 2

Clinical chart of patient with moderately severe atypical pneumonia. Roentgenograms show early hilar infiltration.

TABLE 4

Distribution of Pulmonary Lesions in 16 Cases of Experimentally Induced Atypical Pneumonia

NUMBER OF LOBES	LOCATION	NUMBER OF PATIENTS
One	Left lower	4
	Right lower	1
Two	Right and left lower	7
More than two	Right upper, middle, lower and left lower	2
	Right upper, middle, lower and left upper and lower	2

The *development* of the pneumonic lesions showed considerable variation. In four patients infiltration was limited to the peripheral portions of one or both lower lobes and the hilar regions were not

abnormal at any time. In six patients the earliest lesion, peribronchial infiltration, appeared to spread centrally toward the hilus with concomitant radial spread within the lower lobe. In one patient of this group, the early lesions spread rapidly in diffuse fashion to involve all 5 lobes. In the remaining six patients perihilar infiltration became increasingly apparent and spread occurred to the peripheral portion of one or more lobes in a wedge or fan-shape with the greatest density of the lesion at the hilus.

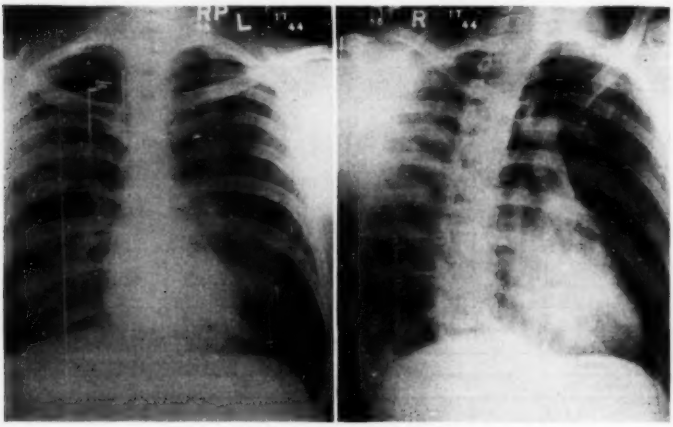


FIG. 6, CASE NO. 45

Roentgenograms of patient with moderately severe atypical pneumonia showing characteristic soft patchy infiltration.

The *distribution of the pulmonary lesions* conformed to that usually observed in the natural disease (Table 4). One or more of the lower lobes was the sole site of infiltration in 75 per cent of the cases. In the remaining 25 per cent the lower lobes were involved in association with lesions in other lobes. In two patients infiltration was present in 4 lobes, and in 2 other patients diffuse lesions were detected in all 5 lobes.

The *duration* of roentgenographic signs was variable. In three patients who were mildly ill the lesions were transitory for 1 to 3 days. In ten patients infiltration was visible for at least one week. The average duration of radiographic signs was approximately 10 days. *Resolution* proceeded in one of two ways, namely, gradual clearing of the lung from the periphery toward the hilus with reduction in size

of the lesion, or a progressive decrease in density of the lesion as a whole. Generally, both methods of clearing went along simultaneously. Rarely, resolution proceeded by gradual clearing from the hilus to the periphery.

Pleural effusion of moderate extent was demonstrated radiographically in one patient. "Plate-like" atelectasis was observed during convalescence in another patient. Persistence of abnormal bronchovascular markings was not observed in any of the patients.

The time of appearance of certain lung signs, as determined by physical examination, and the development of roentgenographic infiltration is shown in Table 5. Fine rales were first detected over a period varying from the 2d to 9th days and coarse rales were audible

TABLE 5

Time of Appearance of Fine and Coarse Rales and Roentgen Signs in 16 Cases of Experimentally Induced Atypical Pneumonia

SIGN	NO. OF CASES	DAY OF DISEASE FROM ONSET OF DEFINITE ILLNESS										
		1	2	3	4	5	6	7	8	9	10	11
Fine rales	15*	0	1	2	5	2	2	1	1	1	0	0
Coarse rales	15*	1	1	6	1	2	1	0	2	0	0	1
Roentgenographic signs	16	0	2	4	7	3	0	0	0	0	0	0

* One patient developed no rales at any time.

for the first time between the 1st and 11th days. Roentgenographic signs, on the other hand, became "positive" between the 2d and 5th days of disease with the maximum number occurring on the 4th day.

No correlations were found in this group of patients between the types of inocula (filtered or untreated) and 1) the location, 2) the extent and 3) the radiographic appearance of the pneumonic lesions. The presence or absence of early hilar involvement were not correlated with the extent of the lesion or severity of infection.

Temperature, Pulse and Respiratory Rates. All of the patients with pneumonia developed fever of variable degree during the course of illness. Fever of 99 degrees or above was present for an average of 9.3 days; fever of 100 degrees or above for 6.3 days. Maximum temperatures varied between 100.2 and 104.8 degrees, the average being 102.8 degrees.

The febrile response varied somewhat according to the type of inoculum. Figure 7 shows the median febrile curves of 7 patients who received filtered inoculum and of 6 individuals inoculated with untreated inoculum. Although the data are limited because of the small number of cases in each group, and, therefore, definite conclusions cannot be drawn, the early febrile course was similar in both groups, the fourth day marked the beginning of the fastigium and the febrile response in those receiving filtrate was of shorter duration, possibly indicative of a milder infection.

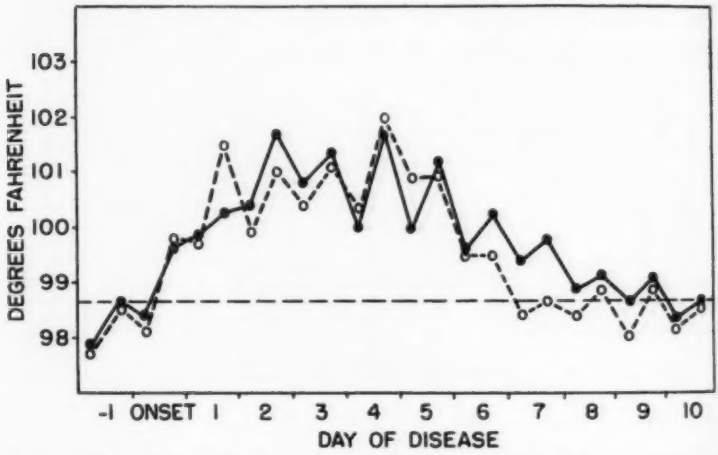


FIG. 7. Median febrile curves in experimentally induced primary atypical pneumonia. Solid lines (untreated inoculum); dotted lines (filtered inoculum).

Of the 26 patients with minor respiratory illness 17 developed fever of 99 degrees or above for an average period of 3 days. In only 5 patients did the temperature exceed 100 degrees. The maximum temperature varied between 99 and 101.2 degrees, the average being 99.5 degrees.

The pulse and respiratory rates in atypical pneumonia were not strikingly elevated during the febrile course, except in a few patients who were severely ill with extensive pneumonic infiltration. The average pulse and respiratory rates for each degree of fever during the

period of clinical illness are given in Table 6. It is apparent that for each degree rise in temperature there was a corresponding increase in pulse and respiratory rates, but that even with higher degrees of fever the rates were not appreciably elevated. In Table 7 the relation of

TABLE 6

Relation of Temperature to Pulse and Respiratory Rates During Clinical Course of Pneumonia*

DEGREES OF TEMPERATURE	NO. OF CASES	NO. OF OBSERVATIONS	AVERAGE PULSE RATE	AVERAGE RESPIRATORY RATE
98–98.9	16	124	74	18
99–99.9	16	158	81	21
100–100.9	16	148	85	21
101–101.9	15	111	89	22
102–102.9	14	101	94	25
103–103.9	8	45	99	25
104–104.9	4	10	106	23

* Clinical course arbitrarily defined as the period between day of onset of definite illness and the first day temperature was below 100°, inclusive.

TABLE 7

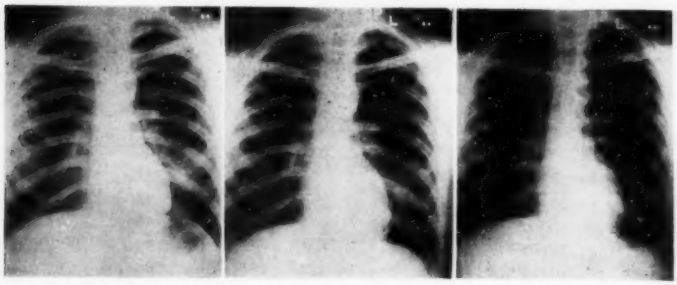
Relation of Temperature to Pulse and Respiratory Rates in Atypical Pneumonia and Pneumococcal Pneumonia

CLINICAL DIAGNOSIS	SOURCE OF DATA	NO. CASES WITH FEVER	AVERAGE MAXIMUM TEMPERATURE (DEGREES F.)	AVERAGE INCREASE IN PULSE RATE FOR EACH DEGREE OF FEVER (BEATS PER MIN.)	AVERAGE INCREASE IN RESPIRATORY RATE FOR EACH DEGREE OF FEVER (RESPIRATIONS PER MIN.)
Atypical pneumonia.....	Experimental	16	102.8	4.9	1.6
Atypical pneumonia.....	Camp Claiborne	277	102.6	4.8	1.1
Pneumococcal pneumonia...	Camp Claiborne	64	104.0	7.1	2.0

temperature to pulse and respiratory rates in atypical pneumonia and pneumococcal pneumonia are contrasted. The data obtained from experimental cases of atypical pneumonia agreed closely with those found in the naturally-acquired disease (3) and also differed from cases of pneumococcal pneumonia.

CLINICAL TYPES OF DISEASE

Atypical Pneumonia. Examples of mild, moderately severe and severe forms of uncomplicated pneumonia were observed among these patients. The illnesses of eight patients were of mild degree;



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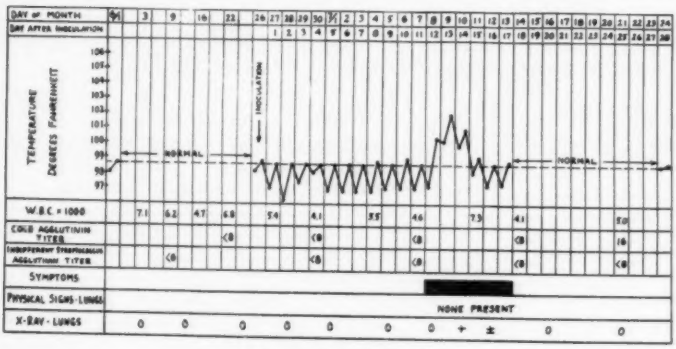


FIG. 8, CASE NO. 25

Clinical chart of patient with mild atypical pneumonia (filtered inoculum).

in five they were moderately severe; and in three the infections were severe.

Mild Illnesses. In this group the symptoms were of mild degree, abnormal physical signs in the lungs were of minimal extent, the febrile course was of short duration, and pulmonary infiltration was confined to one or both of the lower lobes. An illustrative example is presented in the following case summary.

Case No. 25 (Figure 8). A 27-year-old male noted abrupt onset of headache, malaise, weakness and slight cough 12 days after receiving filtered inoculum. Roentgenogram on the 14th day (July 10th) showed exaggeration of the hilar shadows and a soft patchy infiltration of the left lower lobe which rapidly cleared over the course of three days. The febrile course lasted three days. Except for headache, the symptoms were not severe and these diminished over a period of 6 days. No abnormal physical signs in the lungs were detected at any time. The leukocyte count was normal. Cultures of the throat revealed normal flora. Neither cold agglutinins nor agglutinins for streptococcus MG were detected in samples of convalescent sera.

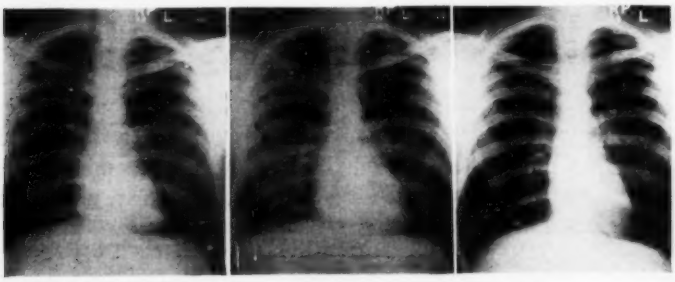
Moderately Severe Illnesses. Patients in this group noted symptoms over a longer period. The febrile course was more protracted and infiltration of 2 or more lobes was detected by physical examination and roentgenogram. Two examples of this type of illness are given in the following case summaries and Figures 5 and 9.

Case No. 2 (Figure 5). This patient, 30 years old, observed chilliness, feverishness, headache, malaise, nasal discharge and sore throat 8 days after inoculation with filtrate. During the next 2 days fever increased in amount. On the 10th and 11th days (July 6 and 7th) the persistence and severity of headache, cough, sputum, chest pain and fever indicated a moderately severe illness. Signs of pulmonary infiltration were first detected on the 11th day at the base of the right lung. The pneumonia ultimately involved the entire right lung and the left lower lobe. The febrile course lasted 7 days. Symptoms and lung signs persisted for approximately three weeks. During convalescence, transient "plate-like" atelectasis was demonstrated by roentgenogram. The leukocyte and differential counts showed mild elevation during the height of disease and on the 22d day. The erythrocyte sedimentation rate (Rourke-Ernstene) was normal. Streptococcus MG was recovered from throat and sputum cultures from the 11th to the 18th days following inoculation. Cold hemagglutinins developed to a titer of 128, but agglutinins for streptococcus MG were not demonstrable.

Case No. 45 (Figure 9). This volunteer, aged 28, noted transient upper respiratory symptoms between the 5th and 8th days following inoculation of filtered material. On the 12th day, definite symptoms of a moderately severe illness became apparent with the development of chilliness, feverishness, headache, malaise and anorexia. The following day the patient noted onset of cough with mucopurulent sputum and the constitutional symptoms continued. Remittent fever, varying between 100 and 103 degrees, persisted for 10 days. Patchy pneumonic infiltration of the entire right lung and left lower lobe were demonstrable in roentgenograms. Subscrepitant rales were first detected on the 20th day and persisted for 8 days. Defervescence was by lysis over a period of 3 days. Convalescence was uneventful.

The leukocyte count showed no elevation. Elevation of the erythrocyte sedimentation rate was not observed at any time. Streptococcus MG was not isolated from throat and sputum cultures. Cold hemagglutinins were present in a maximum titer of 256 on the 25th day after inoculation, or 13 days after onset of illness. Streptococcus MG agglutinins increased to a titer of 16 at the same time and reached a titer of 32, 4 weeks later.

Severe Illnesses. Two patients developed severe illnesses in association with extensive pneumonic infiltration. In one additional patient the infection was complicated by disorientation and emotional instability.



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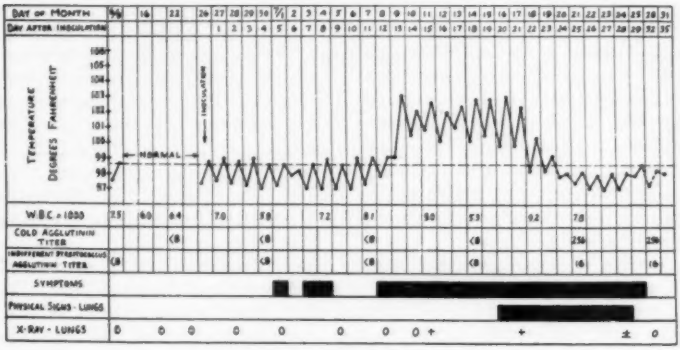

FIG. 9, CASE NO. 45

Clinical chart of patient with moderately severe atypical pneumonia (filtered inoculum).

Case No. 4 (Figure 10). A 29-year-old volunteer received untreated inoculum. Seven days later he observed mild sore throat and hoarseness. The following day (July 4th) definite symptoms of illness were apparent with the development of headache, feverishness, malaise, nasal obstruction, dry cough and chest pain. The temperature was 100 degrees and physical examination showed no abnormal lung signs. During the course of the next three days he became progressively sicker with severe frontal headache, paroxysmal cough, substernal pain and high fever. Pulmonary infiltration appeared first in the right upper

and left lower lobes and ultimately extended to involve all five lobes. Roentgenograms at first showed small, soft patchy lesions of irregular size and shape; subsequently these appeared nodular and discrete and were scattered diffusely throughout both lungs. Later, the nodular lesions became confluent but the hilar regions showed no striking involvement. Oxygen therapy was administered for approximately one week because of cyanosis and the critical nature of illness. The patient improved with the gradual clearing of the pneu-

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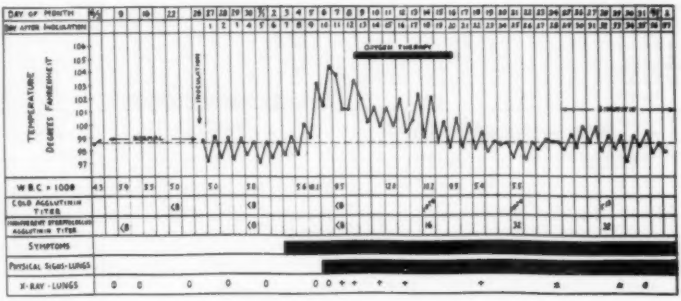


FIG. 10, CASE NO. 4

Clinical chart of patient with severe atypical pneumonia (untreated inoculum).

monic process. Convalescence was complicated by an acute maxillary sinusitis, but recovery was ultimately complete.

The total leukocyte and polymorphonuclear cell counts were moderately elevated during the febrile period. The corrected erythrocyte sedimentation rate showed maximum elevation of 1.2 mm. per minute. Sputum cultures showed pneumococci, types 3 and 11, and streptococcus MG. Cold hemagglutinins and streptococcus MG agglutinins reached titers of 1024 and 32, respectively, in the convalescent sera.

Minor Respiratory Illness. As indicated in the second paper (1b), variability was noted in the clinical types of minor respiratory illnesses encountered among 26 patients. Three illustrative examples are given in the following case summaries.

Case No. 9, 31 years old. This individual received untreated inoculum and noted onset of mild respiratory symptoms on the 4th day after inoculation. Between the 4th and 9th days the principal symptoms were headache, sore throat, nasal discharge, hoarseness and dry cough. Physical examination revealed no abnormal findings in the upper respiratory passages or lungs. Roentgenograms showed no evidence of pulmonary infiltration. Fever was not present at any time. Pharyngeal cultures showed normal bacteriological flora before and after inoculation. The leukocyte count and erythrocyte sedimentation

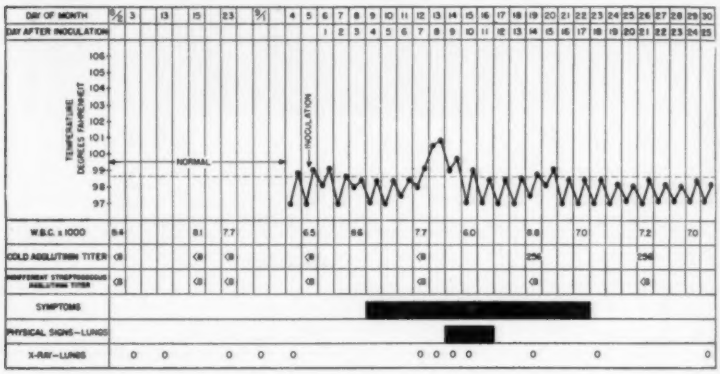


FIG. 11, CASE NO. 131

Clinical chart of patient with minor respiratory illness (untreated inoculum).

rate were normal. Cold hemagglutinins were present in a titer of 8 in convalescent serum specimens. Streptococcus MG agglutinins were not found.

Case No. 131 (Figure 11). This 19-year-old male was well until 4 days after inoculation with untreated material. Nasal obstruction and discharge and dry cough were the earliest symptoms noted but these were unaccompanied by fever or abnormal physical signs. On the 8th day (September 13th) he experienced several shaking chills, feverishness, and headache and the cough became aggravated. With rise in temperature to 100.6 degrees he appeared moderately ill. Between the 10th and 12th days fine rales were audible over the left lower lobe. The febrile course was of short duration. Roentgenographic abnormalities of the lungs were not found at any time. The leukocyte count and sedimentation rate were normal. Throat flora was normal. Cold hemagglutinins were present in a titer of 256 on the 14th day after inoculation. Streptococcus MG agglutinins were not found.

Case No. 134 (Figure 12). Ten days after inoculation of filtrate this individual noted gradual onset of sore throat and hoarseness. Moderate injection and hypertrophy of the

pharyngeal lymph follicles were the only abnormal physical signs noted at this time. On the 12th day (September 9) a short febrile illness began which was accompanied by chilliness, feverishness, malaise, cough and sputum. Fine rales and rhonchi were audible over the right lower lobe. Roentgenograms showed prominent hilar markings on the right but no evidence of pneumonic infiltration. Convalescence was uneventful.

The leukocyte count rose slightly during the febrile period but was under 10,000 at all times. Throat cultures showed normal flora. Cold hemagglutinins reached a titer of 64 on the 21st day, 11 days after onset of symptoms. Streptococcus MG agglutinins were noted in low titer, 8, on the 28th day.

Comment. Case No. 9 represents an extremely mild type of illness in which symptoms and signs of respiratory infection were minimal. Cases 131 and 134 illustrate minor febrile illnesses of more severe

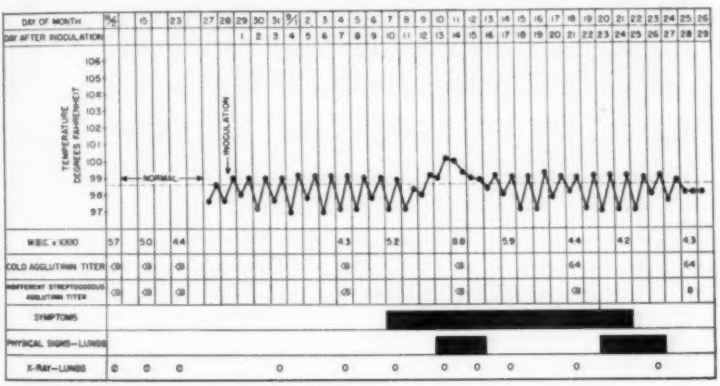


FIG. 12, CASE NO. 134

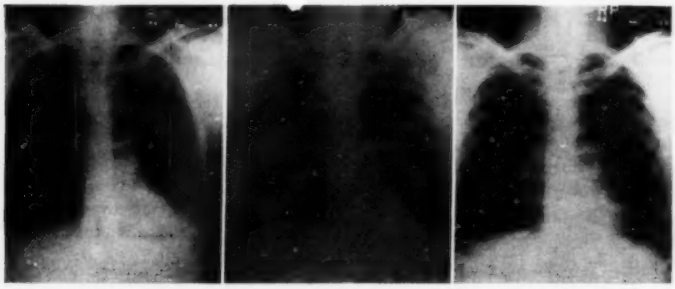
Clinical chart of patient with minor respiratory illness (filtrate).

degree which resulted from the inhalation of untreated and filtered inoculum, respectively. Except for the absence of positive roentgenograms the illnesses of these latter two patients resembled primary atypical pneumonia.

COMPLICATIONS

Complications were observed in four patients with atypical pneumonia. Case No. 4 (Figure 10) developed maxillary sinusitis during the recovery period. Case No. 24, a severe illness, was complicated by toxic psychosis during the febrile period. The chief manifestations

of this complication were disorientation and emotional instability. Neurological examination revealed no abnormal findings and the cerebrospinal fluid was normal. Recovery was complete. Pleural



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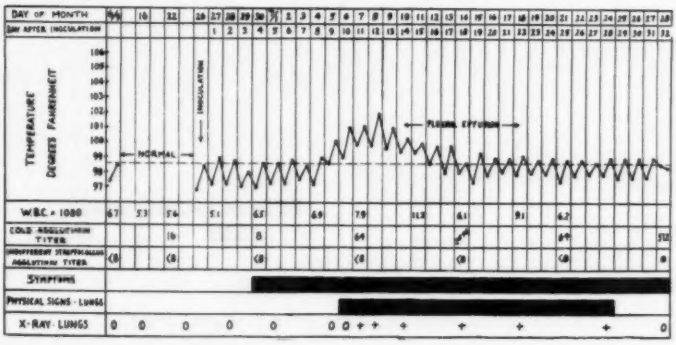


FIG. 13, CASE NO. 44

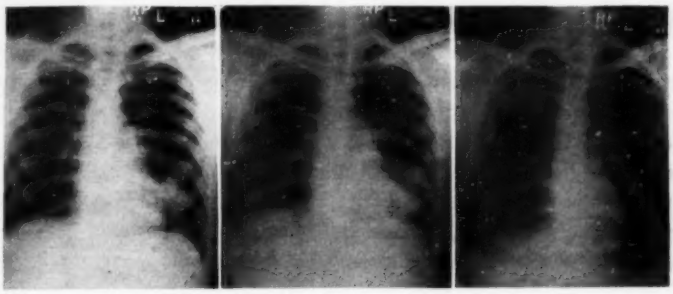
Clinical chart of patient who developed pleural effusion during the course of atypical pneumonia.

effusion and encephalomyelitis each occurred in one case. Summaries of the clinical course of these patients are given below.

Case No. 44 (Figure 13). This patient experienced mild atypical pneumonia beginning 8 days after inoculation of untreated material. Physical signs and roentgenograms indicated bilateral infiltration of the lower lobes. On the 14th day after inoculation, approximately one week after onset of illness, signs of pleural effusion were detected at the left base. Thd fluid was sterile, greenish-yellow in color and contained 1370 leukocytes and

240 erythrocytes per cubic millimeter. Approximately 55 per cent of the leukocytes were large mononuclear cells; the remainder were lymphocytes and neutrohpils. Resorption of fluid was rapid and ultimate recovery complete. No residual roentgenographic signs were present.

Case No. 47 (Figure 14). This patient suffered from a mild atypical pneumonia, apparently contracted by air-borne infection at the time of inoculation. Pulmonary infiltration



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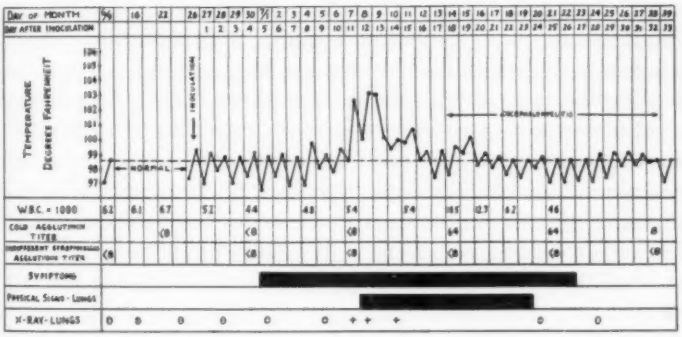

FIG. 14, CASE NO. 47

Clinical chart of patient with mild atypical pneumonia; clinical course complicated by encephalomyetitis.

was confined to the left lower lobe. On the 18th post-inoculation day, symptoms and signs of meningeal irritation appeared. Subsequently, the patient experienced drowsiness, transient paralysis of the bladder and rectal sphincter, and paresthesias of the arms and legs. The abdominal and cremasteric reflexes were absent. Deep tendon reflexes in the legs were exaggerated. Babinski and Oppenheim reflexes were positive on the right. Kernig and Brudzinski signs were negative. Skeletal muscular tone was normal; rectal sphincter tone was diminished. Touch and pain sensibilities to pin-prick were normal.

The cerebrospinal fluid was clear and contained 17 small mononuclear cells per cubic millimeter. Chemical determinations were within normal limits and the fluid was sterile.

The neurological findings suggested a diffuse pathological process. Encephalomyelitis appeared to be the most probable diagnosis. The subsequent course was featured by paresthesias of the legs which continued for approximately two months; at this time neurological examination revealed normal findings and recovery was ultimately complete.

DISCUSSION

Atypical Pneumonia. The results of an analysis of the clinical features of experimentally induced atypical pneumonia have shown that the experimental disease was similar to the naturally-acquired infection. An insidious onset characterized the inception of illness in the majority of the experimental subjects. Constitutional symptoms were conspicuous features of the early stage of illness, and local symptoms referable to the upper or lower respiratory tract were commonly observed. The occurrence of local symptoms prior to the appearance of fever or onset of constitutional symptoms was noted in a few patients. These symptoms were usually persistent but occasionally disappeared altogether before the development of objective signs of illness. They were thought not to be the immediate result of the mechanics of inoculation inasmuch as they occurred after an interval of several days. This observation suggested (*1*) that a prodromal period of infection existed in these patients, or (*2*) that the inocula contained two viruses, one of which induced minor illness and the other atypical pneumonia. The hypothesis that the early stage of atypical pneumonia in some patients is characterized by a prodromal period of infection appears not unreasonable but incapable of proof. Whether this condition exists in the naturally-acquired disease is unknown. Evidence for the existence of two agents in the inocula was not apparent but this possibility cannot be excluded, as was suggested in a previous section of this report (1b).

The physical signs manifested by these patients disclosed moderate inflammatory changes in the upper respiratory tract and varying degrees of infiltration of one or more lobes of the lungs. As noted in the naturally-acquired disease, rales were found over the areas of infiltration in approximately 90 per cent of the cases; all but one patient showed this sign. The time of appearance of rales, however, showed certain differences. Coarse rales, in general, appeared earlier than

fine rales; eight cases developed coarse rales by the third day and the same number of patients developed fine rales by the fourth day. The early appearance of abnormal lung signs in the majority of these patients may be explained by the fact that daily observation of the cases afforded greater opportunity for their early recognition. In some patients, however, the development of rales was a late manifestation of disease, a finding which is usually observed in naturally-acquired infections. In seven individuals fine rales were first detected from the 5th to the 9th day, and in 6 individuals coarse rales first appeared during this time.

The roentgenographic picture of experimental atypical pneumonia was strikingly similar to that of the natural disease. The extreme variability in size, shape and density of the pneumonic lesion was confirmed. However, the earliest roentgenographic sign in the majority of these patients was not perihilar involvement but peribronchial infiltration at the periphery of one or more lower lobes. This early sign was detected repeatedly and sometimes exclusively by anterior-oblique chest films. Such lesions subsequently were either limited in extent, spread centrally toward the hilus within the lobe or spread diffusely throughout one or more lobes. In only 6 patients was perihilar infiltration the earliest roentgenographic sign. Although these observations are limited in extent they suggest that an analogous situation may exist in cases of naturally-acquired infection; namely, that the initial infiltration is primarily peribronchial in type and that hilar involvement or diffuse spread occurs secondarily.

The height and duration of the febrile reaction in these cases showed the same variability that is commonly noted among hospitalized soldiers or civilians with atypical pneumonia. A study of the median febrile curves revealed that the 4th day of disease marked the period of maximum fever and the beginning of the fastigium. Slight differences were found in the shape of the febrile pattern between those inoculated with filtrate and those receiving untreated material, although the small number of cases in each group made definite conclusions impossible.

Comparison of the average pulse and respiratory rates for each degree of fever during the clinical illness resulted in the finding of relative bradycardia and the absence of tachypnea. These objective

data thus confirm the clinical impression held by most observers (2, 3, 4).

The clinical course of the experimental disease was featured by examples of mild, moderately severe and severe forms of illness. Instances of each of the foregoing types of infection were found among those who had filtered and those who received untreated inoculum. Two of three patients who were severely ill received untreated inoculum; on the other hand, three of five patients who were moderately ill were inoculated with filtrate. Limited evidence was thus obtained that untreated inoculum resulted in a slightly more severe degree of infection. It cannot be assumed, however, that the type of inoculum was the sole factor which conditioned the type of infection. Unknown host factors probably played an equally important rôle.

The occurrence of complications in four patients provided additional evidence that these cases resembled those observed of primary atypical pneumonia which are naturally-acquired. The development of clinical and roentgenographic evidence of maxillary sinusitis in one patient suggested secondary bacterial infection; through an oversight cultures were not taken and, therefore, diagnosis was not definitely established. The occurrence of sterile pleural effusion in one of the 16 cases was of interest inasmuch as this complication is generally observed in less than 5 per cent of naturally-acquired infections. In recent years the occurrence of complications of the central nervous system during the course of atypical pneumonia has been reported (5, 6, 7). Baker and Noran (8) have reviewed the recent literature on this subject and have suggested that pneumonia, regardless of the etiologic agent, may be complicated by encephalitis or other involvement of the central nervous system. Two fatal cases of atypical pneumonia have been observed by the Commission (9), both of which at autopsy showed perivascular hemorrhages throughout the brain. It is possible that the encephalomyelitic complication which developed in one of the volunteers had a similar pathologic nature.

Minor Respiratory Illness. The clinical features of minor respiratory illness were those of a mild to moderately severe infectious process of the upper and lower respiratory tract. The diagnosis of this affection was one of exclusion and it was made on all patients who had symptoms or signs of respiratory infection but who did not develop

roentgenographic evidence of pulmonary infiltration. As such, considerable variation was to be expected in the clinical picture. Instances of mild infection apparently limited to the upper respiratory passages, and also instances of moderately severe febrile infection with rales in the lungs, formed the extremes of this diagnostic group. Nevertheless, consideration of symptoms and physical signs of the group as a whole showed many points of similarity to the group which contracted atypical pneumonia. Except for lessened frequency the symptoms in the minor illness group were the same. Physical findings showed lower frequency of involvement of nose, pharynx and lungs. Cervical adenopathy was found in 4 patients; in none of the cases of pneumonia was cervical adenopathy present. Fine and coarse rales were audible in the lungs of 5 patients. The observations indicate, then, that the clinical illnesses of these patients were similar to those of atypical pneumonia except for being more mild. The epidemiological and etiological relationship of these cases to atypical pneumonia has been discussed in a previous paper.

SUMMARY

The clinical features observed in 16 cases of experimentally induced primary atypical pneumonia have been described. The symptomatology, physical findings, roentgenographic pattern and clinical course of disease in these patients were similar to those which are characteristic of the naturally-acquired infection.

An analysis of 26 cases of minor respiratory illness revealed that the symptoms and physical signs, except for lessened frequency, were essentially the same as in individuals who contracted pneumonia. This was particularly evident in a few individuals who experienced moderately severe febrile reaction and had rales in the lungs; it was less commonly observed in those with mild infections which were apparently limited to the upper respiratory passages.

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THE TRANSMISSION OF PRIMARY ATYPICAL PNEUMONIA TO HUMAN VOLUNTEERS*

IV. Laboratory Studies

COMMISSION ON ACUTE RESPIRATORY DISEASES**
Fort Bragg, North Carolina

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The experimental methods, results of inoculation and clinical features of experimentally induced primary atypical pneumonia and minor respiratory illness have been reported in the foregoing papers (1a, b, c). During the course of this investigation a number of laboratory studies were made for the purpose of complementing the clinical picture of the experimental disease and in order to rule out other diseases of known bacterial or viral etiology. A summary of the results of laboratory studies obtained in two experiments forms the subject of this report.

HEMATOLOGICAL STUDIES

Leukocyte Count. The leukocyte count in the cases of pneumonia was, in general, within normal limits although slight elevations occurred in some patients during the course of the disease. Prior to onset the average levels varied individually from 4600 to 8800; for the group of 16 cases the average pre-onset level was 6023. The averages of the individual increases in leukocyte count above their respective pre-onset levels were compared for each 3-day period in all 16 cases (Figure 1). In the first 3-day period the average increase was 1000 cells; in the next two periods the increments were approximately 2000 cells. Between the 12th and 14th days the increment was 1800 cells and thereafter the average count approached pre-onset levels. Thus, at no time following onset did the average increments reveal a pronounced leukocytosis.

The average differential leukocyte count prior to onset was 53 per cent polymorphonuclear neutrophils and 43 per cent lymphocytes. During the first 6 days of disease there was slight increase in average percentage of polymorphonuclear cells from 53 to 67 per cent and a corresponding decrease in percentage of lymphocytes. At no period following onset were neutrophilia, lymphocytosis, eosinophilia or monocytosis observed.

The leukocyte counts in the 26 cases of minor respiratory illness were likewise within normal limits of 5000 to 10,000. The average pre-onset leukocyte count in these cases was 6510 and, after onset, the

averages of the individual increases above their respective pre-onset levels showed a maximum elevation of only 350 cells.

Erythrocyte Sedimentation Rate. In the cases of pneumonia the erythrocyte sedimentation rate showed variation in extent of increase and also in time of appearance. Maximum levels varied between 2 and 41 mm. (uncorrected) and from 0.1 to 1.2 mm. (corrected). Eight of the patients developed maximum levels after the febrile

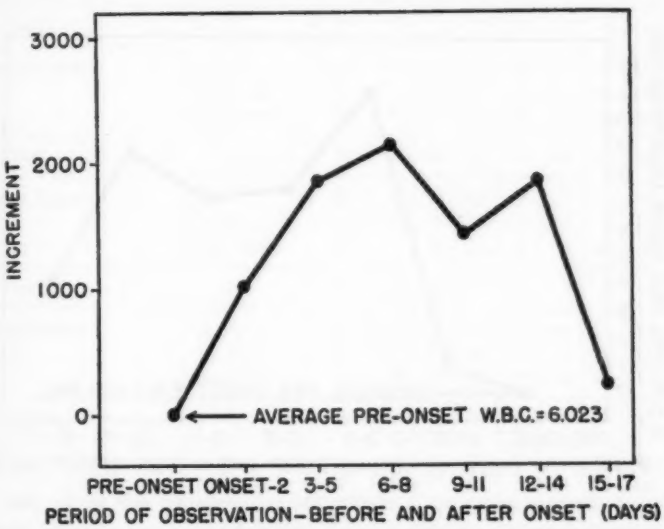

FIG. 1. Average increment in white blood cells in 16 cases of experimentally induced primary atypical pneumonia.

period (less than 100°F.) had ended. Seven patients showed maximum levels during the febrile period; one individual showed no change in rate throughout the entire clinical course. Although the sedimentation rate in some individuals was elevated above normal, the average level of the entire group, when related individually to pre-onset level, showed no striking increase at any time. Figure 2 shows the average increment in uncorrected rate by 3-day periods. The greatest increment occurred from the third to the the fifth day after onset, but

the total increment was not excessive. Definite but slight increases in uncorrected rate were still present as late as the 15th to 17th day.

The erythrocyte sedimentation rate was abnormal (35 mm., uncorrected) in only one individual with minor respiratory illness.

BACTERIOLOGY

Throat Cultures. The results of cultures taken at weekly intervals were analyzed for the frequency of five organisms which are known to

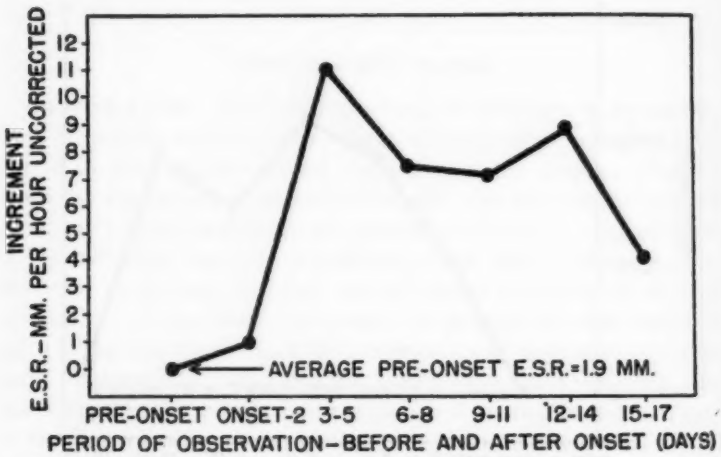

FIG. 2. Average increment in erythrocyte sedimentation rate in 16 cases of experimentally induced primary atypical pneumonia.

be common inhabitants of the respiratory tract. These were β-hemolytic streptococcus, pneumococcus, *Staphylococcus aureus*, *Hemophilus influenzae* and *Hemophilus hemolyticus*. Table 1 summarizes the cultural results in 78 individuals who participated in both experiments. The percentage of individuals showing a given organism at weekly intervals before and after inoculation is presented for each diagnostic group. β-hemolytic streptococcus was isolated from a small proportion of the total group before inoculation, and the latter procedure was not followed by a significant increase in recovery of this organism. Seventeen individuals at some time during observation were found to

TABLE 1

Frequency of Isolation of Certain Organisms Before and After Inoculation in Relation to Type of Illness

ORGANISM	DIAGNOSTIC GROUP†	NO. OF INDIVIDUALS	WEEK BEFORE INOCULATION*				WEEK AFTER INOCULATION*			
			4	3	2	1	1	2	3	4
Beta-hemolytic streptococcus	A. P.	16	10	0	0	7	0	0	7	6
	M. R. I.	26	8	0	8	0	7	4	4	20
	No illness	36	3	2	9	6	5	6	3	6
Total		78	6	1	7	4	5	4	4	11
Pneumococcus	A. P.	16	36	46	46	64	35	37	42	33
	M. R. I.	26	32	44	33	38	38	44	32	16
	No illness	36	43	48	58	48	52	60	41	52
Total		78	38	46	47	48	44	50	38	36
Staphylococcus	A. P.	16	10	20	21	7	0	6	0	13
	M. R. I.	26	20	9	13	12	3	8	16	8
	No illness	36	9	11	22	12	8	12	0	3
Total		78	13	12	19	11	5	9	5	7
H. influenzae	A. P.	16	60	40	42	23	64	25	57	46
	M. R. I.	26	32	40	39	36	53	50	72	45
	No illness	36	29	35	51	12	54	33	33	37
Total		78	34	38	45	22	56	36	52	42
H. hemolyticus	A. P.	16	60	46	35	30	85	56	50	40
	M. R. I.	26	56	27	43	48	69	50	64	37
	No illness	36	70	47	35	27	48	48	60	50
Total		78	63	40	38	35	62	50	59	43

* Results are expressed as per cent of cultures which were positive.

† A.P. = atypical pneumonia; M.R.I. = minor respiratory illness.

harbor β-hemolytic streptococcus of the following groups: Group A, 10 patients; Group C, 2; Group F, 1; and Group G, 2 patients.

The frequency of recovery of pneumococci showed no striking differences in the various diagnostic groups either before or after inoculation. Single or multiple types of pneumococci were recovered from 61 volunteers at some time during the cultural survey; in 17 individuals pneumococcus was not isolated. The type distribution conformed to

that usually observed in large groups of individuals without illness (2). In order of frequency some of the more common types encountered were 3, 8, 4, 6, 19, 18, 22, 9, 17, 20 and 23.

Staphylococcus aureus was present in from 11 to 19 per cent of individuals before inoculation and in approximately 5 to 10 per cent after inoculation. *H. influenzae* was isolated in greater frequency in the first culture following inoculation in each of the diagnostic groups. Individuals who had atypical pneumonia or minor respiratory illness, showed a similar increase in frequency on the third culture, in contrast to those in the "no illness" group. *H. hemolyticus* was recovered from the majority (56–70 per cent) of individuals when they were first cultured (4 weeks before inoculation). During the period of subsequent observation there was, in general, a gradually decreasing percentage of individuals in each of the diagnostic groups showing this organism. The first culture after inoculation disclosed an increase in frequency of *H. hemolyticus* in the atypical pneumonia group and also definite but less marked increase in the other two groups.

Nose Cultures. Weekly cultures of the nasal mucosa of each volunteer revealed normal flora. *Staphylococcus aureus* was the commonest organism encountered. Pneumococcus, type 22, was isolated from one patient. β-hemolytic streptococcus was not present in nasal cultures from any of the volunteers.

Cultures of Sputum and Throat Washings. During the period of febrile illness routine aerobic cultures of sputum or throat washings or both were performed on 14 patients, 10 with atypical pneumonia and 4 with minor respiratory illness. These cultures failed to reveal the presence of organisms which seemed to be causally related to atypical pneumonia or to minor respiratory illness.

Blood Cultures were taken during the early febrile period from 9 patients with atypical pneumonia. All of these cultures remained sterile after prolonged incubation.

Cultural and Serological Studies with Streptococcus MG. Intensive cultural efforts were made to recover streptococcus MG from each volunteer. The special techniques recommended by Mirick and co-workers (3) were followed closely in order to observe the distribution of this organism in the throat cultures, sputum and throat washings from the volunteers before and after inoculation. Evidence was

thus obtained bearing on the possible etiologic significance of this organism in primary atypical pneumonia. The results of these bacteriological studies in 16 individuals who subsequently developed pneumonia are given in Table 2. It will be seen that streptococcus MG was present in 5 and absent in 11 individuals before inoculation. After inoculation, it was present in 7 and absent in 9 persons. The organism was isolated from 3 volunteers both before and after inoculation; it was not recovered from 7 individuals either before or after inoculation; in 6 cases streptococcus MG was isolated only during one period, that is either before or after.

The results of cultural studies on the entire group of 78 volunteers

TABLE 2

*Number of Cases of Atypical Pneumonia from which Streptococcus MG was Isolated in Relation to Time of Inoculation**

BEFORE INOCULATION	AFTER INOCULATION		
	Present	Absent	Total
Present	3	2	5
Absent	4	7	11
Total	7	9	16

* Based on cultures of throat swabs, sputa and throat washings.

were as follows. Streptococcus MG was isolated from 21 persons before inoculation and from 22 after inoculation; it was not isolated from 57 and 56 individuals before and after inoculation, respectively. It is apparent that this organism was isolated with almost identical frequency both before and subsequent to inoculation. As noted in Part II of this report (1b), a rise in titer of agglutinins for streptococcus MG was observed in the sera of only three individuals. Two of these patients had atypical pneumonia and one had minor respiratory illness. Fourteen persons with atypical pneumonia and 25 with minor illness failed to show a detectable rise in agglutinins following inoculation.

Cold Hemagglutination. A 2-tube or greater rise in titer of cold hemagglutinins following inoculation was demonstrated in the sera of 24 of the total number of 78 volunteers in the two experiments (Table

3). Four additional cases showed a rise of only one tube. The total frequency of 2-tube rises varied slightly from one experiment to the other; differences in incidence were found also according to clinical diagnosis.

Among the 36 subjects who participated in the second experiment 9 showed a 2-tube or greater rise in cold hemagglutinin titer, 2 showed a one-tube rise and 25 individuals had no change in titer after inoculation. Eight of 9 individuals with 2-tube rises had atypical pneumonia and one had minor respiratory illness. Two individuals whose sera

TABLE 3

Individuals with 2-Tube or Greater Rise in Titer of Cold Hemagglutinins According to Clinical Diagnosis

CLINICAL DIAGNOSIS*	SECOND EXPERIMENT		THIRD EXPERIMENT	
	Total cases	Number showing 2-tube rise	Total cases	Number showing 2-tube rise
A.P.	10	8	6	5
M.R.I.	15	1	11	9
No illness	11	0	25	1†
Total	36	9	42	15

* A.P. = atypical pneumonia; M.R.I. = minor respiratory illness.

† Case No. 132: Untreated inoculum. Titer of 16 on 14th day after inoculation (inapparent infection).

showed 1-tube rises in titer had no detectable illness. Each of these two received autoclaved inoculum. The rise in titer occurred on the 11th day in one case and on the 25th day in the other. The validity of these observations, however, is open to question in view of the fact that cross infection is believed to have occurred at the time of inoculation. Technical variations also may have accounted for the findings.

Analysis of the results of the second experiment according to clinical diagnosis revealed that of the 10 pneumonia patients, 8 showed significant cold hemagglutinins and 2 had no change in titers. Of the 15 individuals with minor illness, only one had a 2-tube increase and 14 showed no change.

In the third experiment there were 42 subjects of whom 15 showed a

2-tube or greater increase in cold hemagglutinin titer, 2 had a 1-tube rise and 25 showed no change in titer after inoculation. Five of the 15 cases with 2-tube rises had atypical pneumonia, 9 had minor illness and one individual (Case No. 132) was well throughout. The pre-inoculation titer of serum from this subject was less than 8 in each of 4 specimens. On the 14th day after inoculation with untreated material the titer was 16 whereupon it decreased to 8 and then to less than 8 in successive weeks. It is suggested that this individual may have had an "inapparent" infection. Of the 2 subjects showing 1-tube rises, one had minor illness and one had no illness. The former (Case No. 124) received filtered inoculum and had a titer of 8 on the 27th day after inoculation. The other individual (Case No. 117) received autoclaved inoculum; sera showed identical titers of 32 in each of 4 specimens between the 7th and 27th days. It is possible that technical variations may have accounted for the 1-tube rises in these two individuals, particularly in the latter case.

When the results of the third experiment are considered according to clinical diagnosis 5 of the 6 pneumonia patients showed significant rises in cold hemagglutinin titer, a finding which approximated that of the preceding experiment. In those with minor illness the results showed a higher proportion of individuals with cold hemagglutinins than had been found in the previous experiment. Nine of 11 patients showed a 2-tube or greater rise in titer. The significance of this observation is unknown.

The highest cold hemagglutinin titers were found in patients with atypical pneumonia (Figure 3). In the group of 13 patients who showed increases, the maximum titers varied between 16 and 8192 with 9 individuals having titers of 128 or greater. Among the 26 patients with minor illness, 11 of whom showed increases, the maximum titers were 256 or below. Four individuals from among the group of 36 without illness, had titers varying between 8 and 32.

Previous reports (4, 5) have shown that cold hemagglutinins begin to appear during the second week of illness in most cases of atypical pneumonia. Maximum titers are usually attained between the 11th and 24th days. The present experimental data reveal that maximum titers in 17 of the 28 cases occurred between the 21st and 27th days after inoculation (Table 4 and Figure 4). If the data, however, are

related to onset of illness, maximum titers were found to occur earlier; 6 patients developed maximum titers during the first week, 11 in the 2nd week and 7 in the 3rd week between the 15th and 19th day.

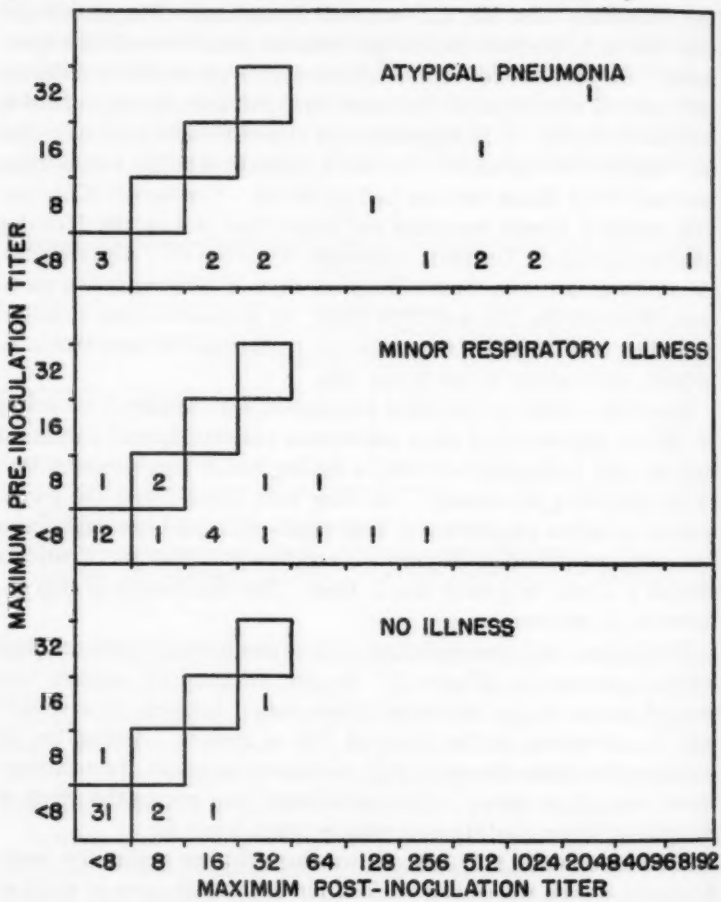

FIG. 3. Pre- and post-inoculation titers of cold hemagglutinins in 78 cases according to clinical diagnosis.

Correlation of maximum cold hemagglutinin titers with extent of pulmonary involvement, height and duration of fever and other indices of severity of illness showed no constant trends. In general, indi-

viduals with extensive pulmonary infiltration had higher titers, but the correlation was by no means complete.

Antibody Tests for Influenza Viruses A and B. Agglutinin-inhibition tests, modified after the method of Hirst (6), were performed on paired sera obtained from each of the 78 volunteers and 12 members of

TABLE 4

Time of Development of Maximum Titers of Cold Hemagglutinins According to Clinical Diagnosis

DAYS AFTER INOCULATION	NO. OF CASES	DIAGNOSIS		
		A.P.	M.R.I.	No illness
7–11	4	0	2	2
14–18	7	2	4	1
21–25	11	9	1	1
27	6	2	4	0

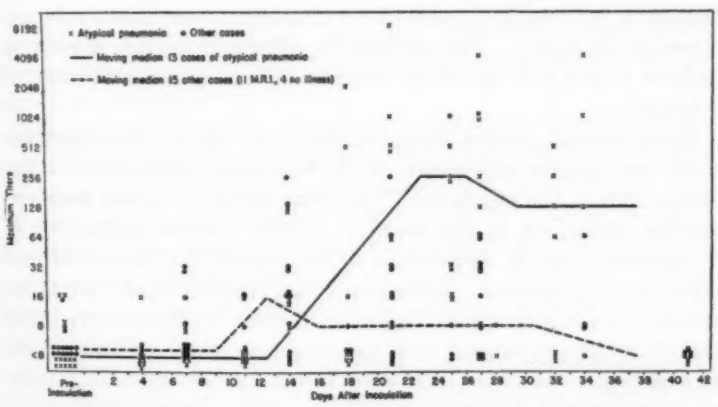

FIG. 4. Cold hemagglutinin titers in 28 cases by days after inoculation.

the staff. The PR8 strain of influenza virus A and the Lee strain of virus B were employed with each set of serum. In no instance was a 4-fold or greater increase in antibody titer to either virus demonstrable. Thus, there was no evidence that influenza virus A or B was present among the group of volunteers or staff and that any of the illnesses

which developed from the inoculations resulted from these specific viruses.

Antistreptolysin Tests. Paired sera, obtained from each volunteer before and approximately 3 to 4 weeks after inoculation, were tested for antibodies to streptolysin "O" by a modification of the method of Hodge and Swift (7). In no instance was a diagnostic increase in antistreptolysin titer considered to be present, although β-hemolytic streptococcus was isolated from throat swabs of 17 individuals. This organism was thus not causally related to the illnesses in any of the volunteers.

DISCUSSION

The experimental cases of pneumonia showed no striking elevation of the leukocyte count when averages of the individual counts in relation to pre-onset level were considered. Furthermore, the secondary rise in total count, reported by Longcope (8) and by Kneeland and Smetana (9) and others was not consistently observed. No striking changes in differential formula of the leukocytes were noted with any degree of constancy. The erythrocyte sedimentation rate showed no uniform trends and was of no value in indicating probable onset of infection.

Bacteriological studies indicated that none of the five organisms which are common inhabitants of the respiratory tract played a significant rôle in the infections. They were present in usual numbers. Possible exceptions to this were the slightly greater frequencies of *H. influenzae* and *H. hemolyticus* in the majority of throat cultures taken after inoculation. Subsequent observations made during an attempt to transmit minor respiratory illnesses to isolated groups of human volunteers showed that the frequency of *H. influenzae* and *H. hemolyticus* varied but little when throat cultures were taken every other day over a 2-month period (10).

The cultural and serological studies employing streptococcus MG and related strains failed to indicate that this organism was causally related to any of the pneumonic illnesses. The occurrence of Streptococcus MG in the throats of individuals both before and after inoculation and in those without illness as well as those with illness, suggests

that this organism may have been a secondary invader. That it may be of possible significance in some cases of atypical pneumonia is indicated by the occurrence of specific antibodies in two of the experimental subjects.

The finding of cold hemagglutinins in the sera of a high proportion of the patients provided additional evidence of the nature of these infections. Six of the 7 donors of the pooled inoculum utilized in the initial experiment of this series had had maximum titers of 256 or greater in their sera. In the experimental subjects, 13 of the 16 cases showed a 2-tube or greater change in titer between acute and convalescent serum samples, with maximum titer varying between 16 and 8192. Although the etiological significance of this test has not been determined it has proved itself of value in confirming the diagnosis of atypical pneumonia when interpreted in conjunction with clinical findings. The studies reported here provide experimental data in support of this conclusion.

SUMMARY

Cultural, hematological and serological studies were made in 16 cases of experimentally induced primary atypical pneumonia and 26 cases of minor respiratory illness.

Bacteriological studies of throat swabs, sputa, and throat washings revealed that none of the five organisms which are common inhabitants of the respiratory tract played a significant rôle in the infections.

Cultural studies of streptococcus MG and related strains indicated that this organism was isolated with almost identical frequency in the total group of volunteers before as well as after inoculation. Agglutinins for the organisms were present in the sera of 2 of the 16 cases of pneumonia.

The white blood cell count and differential leukocyte formula were within normal limits. The erythrocyte sedimentation rate was elevated in a few cases of pneumonia but in general showed no characteristic pattern. The test was of no value in indicating probable onset of infection.

A rise in titer of cold hemagglutinins was demonstrated in the sera of 13 cases of pneumonia and in 11 of the 26 cases of minor respiratory illness.

No evidence was obtained that influenza viruses A or B were causally related to the illnesses of the volunteers.

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THE EFFECT OF THE METHOD OF ADMINISTRATION ON THE THERAPEUTIC EFFICACY OF SODIUM PENICILLIN IN EXPERIMENTAL SYPHILIS*

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The discovery by Mahoney, Arnold and Harris (1) that repeated injections of penicillin will cure early syphilis has been followed by large-scale clinical studies (2) designed to establish the optimum method for its administration. A number of variables have already complicated that clinical evaluation. The more important of these are a) the use of a suspension of penicillin in peanut oil and beeswax (17) instead of an aqueous solution, b) the possible simultaneous use of mapharsen (3), bismuth or both, to promote therapeutic efficacy, and c) the possibility that the several types of crystalline penicillin present in the commercial product in varying concentrations may differ also in their spirocheticidal activity (4). Even if one limits the problem to the action of commercial sodium penicillin injected intramuscularly in aqeuous solution at least three variables may modify therapeutic efficacy: the number of injections, their frequency, and the total amount of penicillin administered. Given the multiple permutations of these three variables, it is a difficult and time-consuming task to determine the optimum method of treatment by studying the end results in early human syphilis. That study was, however, feasible of much more rapid and accurate evaluation in the experimental animal.

As will be shown in the present paper, the curative dose of sodium penicillin in rabbits has been found to be affected to a striking degree by the number of injections. The greater that number, the less was the *total* amount of penicillin required for cure. On the other hand, provided only that the interval between injections was sufficiently great to avoid cumulative effects on the blood penicillin level (in which case therapeutic efficacy was paradoxically decreased), it made no difference whether penicillin was administered every four hours, twice daily or daily: an equal number of injections at a given dosage level produced equivalent effects.

*This work was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Johns Hopkins University.

The clear implications of these findings with respect to the optimum method for the treatment of human syphilis are discussed in the text. The factors underlying the observed data, and in particular the correlation between the blood concentration of penicillin and its therapeutic efficacy, will be discussed in a following paper (5).

A. METHODS AND MATERIALS

1. *Inoculation and treatment of rabbits:* All the animals used in these studies were inoculated intratesticularly with the Nichols strain of S. pallida, and treatment with sodium penicillin was begun just 5 to 7 weeks later, after the development of testicular syphilomata had been confirmed by darkfield examination. The necessity of keeping this interval constant will be discussed in a following paper (6). The penicillin was injected in aqueous solution into the upper muscles of the hind legs. Thirteen different schedules of treatment were used in which the interval between injections varied form 15 minutes to 4 days, the total number of injections from 4 to 50, and the duration of treatment from 3 hours to 16 days (cf. table 1). On each of these schedules, 3 to 9 rabbits were treated at each of 3 to 7 dosage levels, which usually varied in two-fold steps. The dosage, adjusted to body weight, is expressed in the tables as the penicillin administered in Oxford units per kg. over the entire treatment period.

Nine different brands of commercial penicillin were used, and 18 different lots, varying in purity from 130 to 1025 Oxford units per mg. Ten to 40 rabbits were treated simultaneously with a single lot of penicillin. A second group subsequently treated at the same dosage levels and in the same experiment sometimes received a different lot or even a different brand of penicillin. No clear-cut difference was observed in such cases in the end results of treatment, and they are not distinguished in the tables. This is of significance in relation to the reported presence in some lots of penicillin of an impurity which is actively spirocheticidal (7), as well as the possibility that the several types of crystalline penicillin may vary in their spirocheticidal action.

2. *Evaluation of cure:* Animals in which spirochetes persisted in the testicular syphiloma for more than 72 hours after the completion of treatment, or in which they subsequently reappeared, were adjudged treatment failures. In those animals which healed uneventfully,

TABLE 1

The effect of the frequency and number of injections on the curative dose of sodium penicillin in rabbit syphilis

TREATMENT WITH PENICILLIN		TOTAL DOSAGE	NO. OF ANIMALS	CURED*	FAILED†	% CURED, (AFTER REED AND MUENCH (14))	CURATIVE DOSE, UNITS PER KG.	
Interval between injections	No. of injections						CD_{50}	CD_{99}
		units/kg.						
15 minutes	16	64000	3	0	3	0	>64000	
		32000	5	0	5	0		
		16000	4	0	4	0		
		8000	2	2	0	0		
1 hour	4	64000	2	0	2	0	>64000	
		32000	3	0	3	0		
		16000	3	0	3	0		
		8000	2	0	2	0		
	16	64000	6	1	5	14	>64000	
		32000	5	0	5			
		16000	5	1	4			
		8000	5	0	5			
		4000	2	0	2			
2 hours	16	64000	6	5	1	91	32000	64000
		32000	6	2	4	50		
		16000	4	0	4	25		
		8000	5	2	3	20		
		4000	6	1	5	6		
		2000	3	0	3	0		
4 hours	4	80000‡	3	3	0		60000±	
		32000	1	0	1			
		16000	2	0	2			
	8	64000	7	2	5	38	80000±	
		32000	6	1	5	9		
		16000	4	0	4	0		
		8000	5	0	4	0		
	20¶	20000	7	7	0	100	1600	8000
		10000	7	6	1	96		
		5000	9	6	3	81		
		2000	7	3	4	58		
		1000	9	4	5	38		
		500	9	4	5	18		
		200	2	0	2	0		
	50 8 days	16000	5	5	0	100	360	500
		8000	5	5	0	100		
		4000	6	6	0	100		
		2000	8	8	0	100		

TABLE 1—*Continued*

TREATMENT WITH PENICILLIN		TOTAL DOSAGE	NO. OF ANIMALS	CURED*	FAILED†	% CURED, (AFTER REED AND MUENCH (14))	CURATIVE DOSE, UNITS PER KG.	
Interval between injections	No. of injections						CD_{50}	CD_{99}
		units/kg.						
4 hours—*Cont.*	50—*Cont.*	1000	7	7	0	100		
		500	7	7	0	100		
		250	8	1	7	12		
	8	64000	6	4	2	91	30000	65000±
		32000	15	7	8	55		
		16000	13	3	10	20		
		8000	12	2	10	6		
Twice daily§	16	16000	6	6	0	100	1770	3500
		8000	7	7	0	100		
		4000	7	7	0	100		
		2000	8	4	4	60		
		1000	8	2	6	17		
		500	3	0	3	0		
	4	64000	4	2	2	67	50000±	80000‖
		32000	4	2	2	33		
		16000	3	0	3	0		
		8000	2	0	2	0		
Daily	16	32000	6	6	0	100	4000	13300
		16000	7	6	1	94		
		8000	5	4	1	82		
		4000	7	4	3	50		
		1600	6	1	5	9		
		800	5	0	5	0		
4 days	4	64000	4	3	1	75	50000±	80000±‖
		32000	4	0	4	0		
		16000	2	0	2	0		
		8000	2	0	2	0		

* Popliteal lymph nodes not infectious (2 transfer animals) 6 months after treatment.

† a) Spirochetes in testicular syphiloma persisting for more than 72 hours after treatment, or reappearing at some subsequent date; or b) lymph nodes infectious for normal rabbits 6 weeks or 6 months after treatment.

‡ These three animals received 5 injections instead of 4, to the indicated total of 80000 units per kg.

§ The 46 animals in this experiment included 24 treated with Na-penicillin, 15 treated with Ca-penicillin, and 7 treated with NH_4-penicillin, all in aqueous solution. The results with the 3 preparations were not significantly different, and they are not distinguished in the table.

‖ Estimated.

¶ 5 injections daily instead of 6. 5 a.m. injection omitted each day.

without relapse, the criterion of cure was the infectiousness of the popliteal lymph nodes for normal rabbits six months after the completion of treatment. Both nodes were then emulsified in approximately 1.5 cc. of 50 per cent rabbit serum, and $\frac{1}{3}$ to $\frac{1}{4}$ of the total emulsion was injected into both testes of each of 2 transfer animals. These were kept under observation for a minimum period of 3 months, and suspicious lesions confirmed by the darkfield examination of material obtained on testicular puncture. If no lesion had developed by the end of the observation period, the testes were removed and an emulsion examined by darkfield before the transfer was adjudged negative. In some animals treated with small doses of penicillin, and in which cure was not anticipated, a single lymph node was sometimes transferred into a single animal six weeks instead of six months after treatment. If that early lymph node transfer proved negative, a second node was removed and transferred to 2 animals 6 months after the completion of treatment, as the ultimate criterion of cure (cf. (8)).

B. EXPERIMENTAL RESULTS

The experimental data on all 13 treatment schedules are given in detail in table 1. The results are summarized in table 2, expressed as the total dose of penicillin which cured 50 per cent and 90 per cent of the animals (CD_{50} and CD_{90}, respectively).[1]

[1] The difference between the dose of penicillin which cured a small fraction of the animals and that which cured more than 90 per cent was often significantly greater than was the case with mapharsen. With the latter, the ratio between the doses which cured 10 per cent and 90 per cent of the animals varied between 1.6 and 4.3 in different treatment schedules, and averaged 3.0 (cf. (9)). In the case of penicillin, however, the corresponding ratio between the CD_{90} and CD_{10} levels varied between 2 and 35, with individual values of 8, 35, 2, 6.2, 4.4, 4 and 8.3 in the six schedules in which data were available.

There are at least possible explanations for this difference between penicillin and mapharsen. One is the fact that several different lots of penicillin were often used in a single experiment, possibly varying in their over-all spirocheticidal activity per unit "penicillin" by virtue of their varying contents of penicillins G, F, X and K, or an actively spirocheticidal "impurity" (cf. 7). A second factor contributing to the wide spread between the dose which cured some and that which cured all the animals may be the widely varying rate at which penicillin is excreted by individual animals, and thus, the widely varying periods of time for which it remains at effectively spirocheticidal levels in the tissues (cf. (5)).

These schedules differ with respect to 3 variables: the number of injections, the interval between injections, and the total duration of treatment. To simplify the analysis of the experimental data, the effect of these variables on therapeutic efficacy will be separately considered, i.e.,

TABLE 2

The effect of the frequency and number of injections on the curative dose of sodium penicillin in rabbit syphilis (after table 1)

INTERVAL BETWEEN INJECTIONS	NO. OF INJECTIONS	CURATIVE DOSE, UNITS/KG.	
		CD_{50}	CD_{90}
15 minutes	16	>64000	
1 hour	4	>64000	
	16	>64000	
2 hours	16	32000	64000
4 hours	4	60000±	
	8	80000±	8000
	20	1600	500
	50	360	
Twice daily	8	30000	65000
	16	1770	3500
Daily	4	50000±	80000*
	16	4000	13300
4 days	4	50000	80000*

* Estimated.

1) The interval between injections fixed: effect of varying the number of injections (and thus, the total duration of treatment).

2) The number of injections fixed: effect of varying the interval between injections (and thus, the total duration of treatment).

3) The duration of treatment fixed: effect of varying the number of injections (and thus, the interval between them).

1. *Effect of duration of treatment on the curative dose of penicillin. (Interval between injections fixed and number of injections varied.):* In figure 1, the CD_{50} values of penicillin on various schedules of ad-

ministration have been plotted to show the effect of the number of injections on its therapeutic efficacy. Each curve in that figure relates to a fixed frequency of treatment, whether at 1-hour, 4-hour, 12-hour (±), or 1-day intervals. In each curve the abscissa is the total number of injections, and the ordinate is the corresponding curative dose. It is apparent that the greater the number of injections, the less was the *total* amount of penicillin required to effect cure. Thus, if one compares the 3 schedules in which penicillin was injected at 4-hour intervals (curve•—•in figure 1), as the number of injections was in-

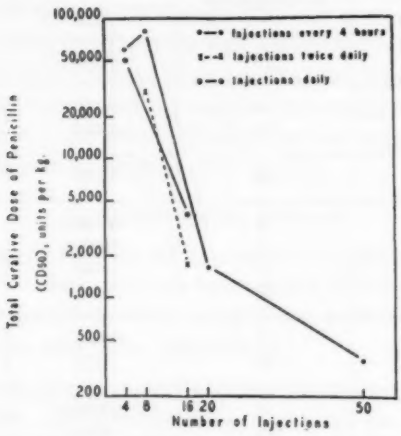

FIG. 1. THE EFFECT OF THE NUMBER OF INJECTIONS ON THE TOTAL CURATIVE DOSE (CD_{50}) OF PENICILLIN (AFTER DATA OF TABLE 2)

creased from 8 to 20 to 50, the total CD_{50} dose of penicillin fell from 80,000 (±) to 1600 to 360 units per kg., approximately a two-hundred-fold increase in therapeutic efficacy. Although not shown in the figure, the CD_{90} dosage of penicillin, the amount necessary to cure 90 per cent of the animals, was correspondingly reduced. An equally striking effect was observed with injections repeated twice daily (curve x—x of figure 1). With eight injections so administered over a period of 4 days, the CD_{50} and CD_{90} dosages were 30,000 and 65,000 units/kg., respectively. Merely doubling the number of injections to 16, thereby increasing the duration of treatment to 8 days, reduced the CD_{50} and

CD_{90} doses to 1770 and 3500 units/kg., respectively, a 20-fold increase in therapeutic efficacy per unit penicillin.

How far this phenomenon can be extrapolated is unknown. It is possible that a schedule involving 100 injections at 4-hour intervals instead of 50, or 32 injections twice daily instead of 16, would have effected a further reduction in the total curative dose. The limiting factor will clearly be that point at which further subdivision of the penicillin results in individual doses so small as not to provide effectively spirocheticidal concentrations for adequate periods of time, and therefore necessitating an increase in the total curative dose. Only further experiments can determine where that limiting factor lies in animal and in man (cf. pages 182 and 185).

On the most efficient method of treatment used in these experiments (50 injections at 4-hour intervals over an 8-day period), all the animals were cured at a total dosage of 500 units per kg., or approximately 0.3 mg. per kg. (assuming the mixture of penicillins present in the commercial product to have an over-all activity approximating that of penicillin G). This is $\frac{1}{10}$th to $\frac{1}{20}$th of the curative dose of mapharsen in the same disease (9). In man, one cannot compare "curative" doses, since neither penicillin (2) (19) nor mapharsen (20) used alone has yet proved capable of curing as much as 80 per cent of the cases of human early syphilis. If one uses as a yardstick for comparison that dosage which cures 70 per cent of the cases, the effective dose of penicillin on a 7-day, 60-injection schedule in man is on the order of 600,000 to 1,200,000 units (2) (19), or approximately 6 to 12 mg. per kg., and that of mapharsen is on the order of 25 mg./kg. (20).

Mg. for mg., penicillin is therefore 2 to 4 times as effective as mapharsen in man, but 10 to 20 times as effective as mapharsen in rabbits. With either drug, rabbit syphilis is much easier to cure than the human disease, requiring less than $\frac{1}{4}$th to $\frac{1}{8}$th as much mapharsen, and less than $\frac{1}{10}$th to $\frac{1}{20}$th as much penicillin. These data, summarized in table 3, emphasize the importance of the host factor in modifying not only the biology of the injection, but also the efficacy of the particular therapeutic agent.

2. *Effect of the frequency of injections on the curative dose of penicillin (Number of injections fixed and duration of treatment varied.)*: In figure 2, the experimental data of table 1 have been replotted to show the effect

of the frequency of injections on the therapeutic efficacy of penicillin. The horizontal variable is now the interval between injections, and the ordinate is again the total curative dose of penicillin (CD_{50}) in units per kg. It is evident from the figure that, *for a fixed number of injections*, the therapeutic efficacy of penicillin was least (the total curative dose a maximum) when the injections were given as frequently as every 15 minutes or 1 hour. As the interval between injections was increased,

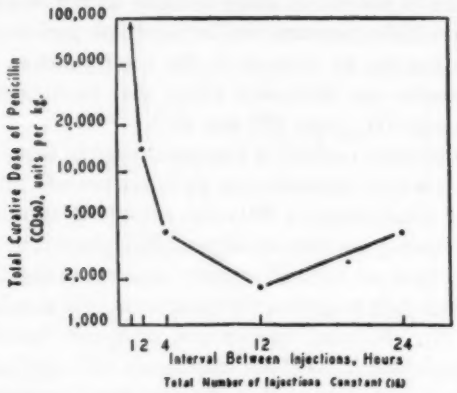

FIG. 2. THE EFFECT OF THE INTERVAL BETWEEN INJECTIONS ON THE TOTAL CURATIVE DOSE (CD_{50}) OF PENICILLIN (AFTER DATA OF TABLE 2)

a) The CD_{50} of 4000 units per kg. for 16 injections repeated at 4-hourly intervals was obtained by interpolation between the CD_{50} values for 8 and 20 injections at that same interval.

b) The 12-hour interval indicated in the figure actually applies to rabbits treated twice daily, with an 8-hour interval between injections.

c) The curative dose for 16 injections at 2-hourly intervals is incorrectly plotted at 14,000 units/kg. instead of 32,000 units/kg. It is clear from the figure that this does not materially affect the shape of the curve.

there was a corresponding increase in therapeutic efficacy, until maximum efficacy was attained with an interval of approximately 8 to 16 hours (cf. legend to fig. 2). Beyond that optimum, and within the limits of the present experiments, further increase in the interval between injections had only a slight effect on the curative dose of penicillin.

Thus, when sixteen injections were given at 1-hourly, 2-hourly and 4-hourly intervals, the curative doses (CD_{50}) were $>64,000$, 32,000 and

(estimated)[2] 4000 units per kg., respectively. Injections at 4-hourly intervals were therefore at least 16 times more effective than injections at hourly intervals. However, further prolongation of the interval between injections to 12 to 24 hours, had only a slight effect, the curative dose on those two schedules being 1770 and 4000 units per kg., tespectively. Whether the difference between the latter values is significant will become evident when data are obtained as to the therapeutic efficacy of 16 injections at e.g. 4-day intervals. If the CD_{50} on the latter schedule proves to be e.g. 16,000 units/kg., then it will be clear that there is a well-defined optimum interval for the administra-

TABLE 3

The curative doses of penicillin and of mapharsen in human and rabbit syphilis

	CURATIVE DOSE IN		RELATIVE "CURATIVE" DOSE IN MAN AND RABBIT
	Man*	Rabbits†	
Penicillin, mg./kg.	6–12	0.3	Man requires 20–40 times as much penicillin
Mapharsen, mg./kg.	25	3–6	Man requires 4 to 8 times as much mapharsen
Relative activity of penicillin and mapharsen	Penicillin 2–4 times more active	Penicillin 10–20 times more active	

* CD_{70} doses; cf. (2)(19)(20).
† CD_{50} doses; cf. (9)(10).

tion of penicillin in experimental syphilis, that interval being on the order of 8 to 12 hours. Injections administered more frequently clearly waste a large portion of the penicillin, and more is required for cure (cf. fig. 2 and fig. 3); with injections administered less frequently, the therapeutic efficacy may also decrease, perhaps because organisms damaged but not killed by the action of penicillin recover sufficiently in the interval between injections to affect the total curative dose (cf. page 183).

3. *Effect of number of injections on the curative dose of penicillin. (Total duration of treatment fixed and interval between injections varied.):* There were 3 schedules in which treatment was completed in a period

[2] Calculated by interpolation from the data of table 2.

of 4 days, but in which the number of injections varied from 4 (once daily) to 8 (twice daily) to 20 (every four hours). The corresponding curative doses (CD_{50}) were 50,000, 20,000 and 1600 units/kg., respectively. The greater the number of injections within a fixed period of time, the greater was the therapeutic efficacy of penicillin, i.e., the less was the total required for cure.

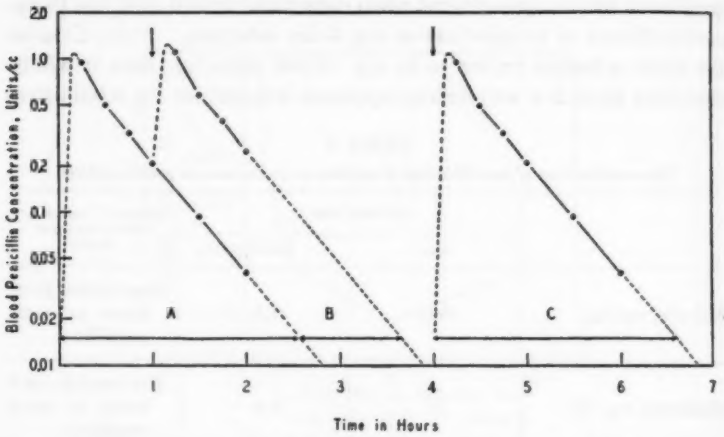

FIG. 3. ILLUSTRATING THE FACT THAT INJECTIONS OF PENICILLIN REPEATED AT WIDE INTERVALS PROVIDE EFFECTIVELY SPIROCHETICIDAL LEVELS FOR A LONGER AGGREGATE TIME (A PLUS C IN THE FIGURE) THAN INJECTIONS REPEATED AT SUCH SHORT INTERVALS AS TO PRODUCE CUMULATIVE EFFECTS ON THE BLOOD PENICILLIN LEVEL (A PLUS B IN THE FIGURE)

The left-hand curve ●—● in the figure is the average blood level obtained in 11 rabbits after a single intramuscular injection of penicillin at 1000 units per kg. The curve ○—○ indicates the anticipated levels were that injection repeated an hour later, when penicillin was still demonstrable at a level of 0.21 units per cc. It is clear from the figure that this lengthens the time for which one has a level of e.g. 0.015 by only 1 hour (cf. B). However, if the injection is repeated 4 hours later, that level is sustained for an additional period of almost 3 hours (cf. C).

As is discussed in a following section, this probably reflects the fact that a low concentration maintained for a long period (many small injections) is more effective than a high concentration acting over a relatively short period (few large injections). The most effective method of administering penicillin would probably be a continuous

intravenous or intramuscular infusion. The present experiments offer no data as to the degree to which the efficacy of penicillin would thereby be increased as compared with e.g. injections repeated at 2-hourly or 4-hourly intervals.

DISCUSSION

Clinical Implications of the Experimental Data

1. ***Mode of action of penicillin:*** The mode of action of penicillin differs from that of the arsenicals in several fundamental respects. When a trivalent arsenical such as mapharsen is injected, it is bound by the organisms in competition with the tissues. The combination is largely completed within a short time, and the spirochetes subsequently die or survive in relation to the amount so bound and their varying individual susceptibility (11) (12). A single exposure to a temporarily high concentration of the arsenical apparently kills as many organisms as repeated exposures to small concentrations. It has been shown in both animals (9) and man (cf. (10)) that the curative dose of mapharsen in syphilis is essentially the same whether the drug is administered in a single massive injection, in 10 to 20 large injections administered over a period of days, or in many small injections administered over a period of months. With penicillin, on the other hand, therapeutic efficacy was enormously enhanced merely by subdividing treatment into a large number of injections. The essential difference seems to be the fact that penicillin is not demonstrably bound and concentrated by the organisms, so that its action depends on the length of time for which effectively spirocheticidal levels are maintained in the body fluids. It is this introduction of the time factor, the necessity for maintaining an effective level, which primarily distinguishes the kinetics of the spirocheticidal action of penicillin from that of e.g. mapharsen.

The therapeutic efficacy of penicillin therefore rests primarily on two factors: its concentration in the body fluids, and the period of time for which the organisms are exposed. Both factors contribute to therapeutic efficacy; but it is the clear implication of the experimental data here reported that of these two variables, the time factor is by far the more important.

2. *Therapeutic significance of the penicillin blood level and the duration of treatment: Relatively low tissue concentrations of penicillin acting over a long period of time (this being the pattern produced by many small injections) were regularly more effective than high concentrations maintained for a short period (the pattern obtained with a few large injections).* The quantitative aspects of this relationship were extraordinary. Fifty 4-hourly injections of 7 units per kg. each, over a total of 8.3 days, were as effective as eight 4-hourly injections of 6000 units per kg. each, over a total of 1.3 days. Dividing the time period and the number of injections by 6 thus required an 850-fold increase in the dosage per injection necessary to produce the same result. Similarly, in animals treated twice daily, if the duration of treatment was halved (8 injections instead of 16) the dosage per injection had to be increased 34-fold in order to effect cure (the CD_{50} dosage per injection 3750 and 110 units per kg., respectively). Even when the total duration of treatment was kept constant, and only the number of injections varied, there was a striking effect on therapeutic efficacy. Thus, in animals treated over a period of 4 days, as the number of injections was increased from 4 to 8 to 20, the total curative dose of penicillin (CD_{50}) fell correspondingly from 50,000 to 20,000 to 1600 units per kg.

These data reflect the fact that penicillin is actively spirocheticidal in extremely low concentration. In vitro, a definite spirocheticidal action has been observed with as little as 0.01 units per cc. (13). In vivo also, when syphilitic rabbits were treated by 50 injections at 4-hour intervals, more than 90 per cent of the animals were cured at dosage levels so small (10 units per kg. per injection) that penicillin was never demonstrable in the blood (less than 0.03 units per cc.). The spirocheticidal action of penicillin is of course enhanced at higher concentrations, but not in proportion to that increase (13); and methods of administration which yield blood levels greatly in excess of the range 0.01 to 0.2 units per cc. waste a large part of the potential activity of the penicillin. The quantitative relationships between the concentration of penicillin and the rate of its spirocheticidal action in vitro will be discussed in detail in a following paper (5).

It follows that schedules of administration which minimize the wasteful peaks in the blood and tissue concentration of penicillin, and provide effective concentrations for maximum periods of time represent

the most efficient use of the drug. Four methods of obtaining this end suggest themselves:

a. The experimental data indicate[3] that the most efficient, if least practicable, method of administering penicillin is a continuous intramuscular, subcutaneous or intravenous drip. Extreme and wasteful variations in the blood and tissue levels would be avoided, and the penicillin could be maintained at a spirocheticidal level for the time necessary to effect sterilization.

b. An alternative approach in the same direction is that suggested by Romansky and Rittman (17), who found that the absorption of penicillin could be delayed by the administration of the calcium salt suspended in peanut oil and beeswax. Wasteful peak concentrations are thereby reduced, and effective levels maintained for longer periods than after the single injection of an equal amount of penicillin in aqueous solution. It must, however, be emphasized that the incorporation of penicillin in oil and beeswax does not shorten the total period of time for which effective levels must be maintained in order to effect cure; it makes it possible to achieve that end with fewer injections, and thus facilitates the possible treatment of syphilis on an ambulatory basis, as discussed in a following section. Sixteen injections of penicillin in oil and beeswax, at a dosage level of e.g. 200,000 units each, admin-

[3] An important reservation must be emphasized when we attempt to apply these experimental data to the treatment of human syphilis. In the rabbit, one determines the most *efficient* method of administering penicillin, that schedule of treatment on which the total curative dose is a minimum; but with any reasonably effective schedule all the animals can be cured if enough penicillin is used. In the human disease, however, with the schedules of treatment hitherto employed, an alarming proportion of early cases relapse even with the largest doses used (19). The therapeutic problem is to find, not the most economic method of administering the drug, but a schedule of treatment on which the proportion of refractory cases is reduced to reasonable levels, and without reference to the amount of penicillin required.

The difference is perhaps more apparent than real. The same amount of penicillin which gives e.g. 15 to 20 per cent failure in early human syphilis when administered by one schedule may well give only 10 per cent failure if administered by a schedule which, per unit penicillin, provides effectively spirocheticidal levels for longer periods of time, and which is therefore several times more effective in the treatment of the experimental disease. At least, this constitutes a reasonable and practical approach to an immediate problem.

istered daily or perhaps even twice weekly, might prove as effective in man as 60 injections of an aqueous solution given at 3-hour intervals at a dosage level of 20,000 units per injection. Experimental data as to the efficacy of the peanut oil-beeswax mixture in a variety of treatment schedules will be described in a following paper (18).

c. Progressive sub-division of the penicillin dosage, keeping the total duration of treatment constant, e.g. 120 injections at 2-hour intervals instead of 60 at 4-hour intervals, is a step in the same direction, leading to lower peak concentrations and the maintenance of effective concentrations for longer periods of time. It has the disadvantage of subjecting the patient to a greater number of injections.

d. The results here reported indicate that prolongation of the treatment period is a highly promising approach to greater therapeutic efficacy. An increase in the number of injections, keeping the interval between them constant, has been shown to effect a striking reduction in the total amount of penicillin necessary for cure (cf. pages 173–175). At least in the experimental animal, and perhaps in man as well, a given amount of penicillin would be far more effective if distributed over a period of 8 days than 4, and would be perhaps more effective if given over e.g. 16 days instead of 8. The longer time period has the further advantage of permitting the simultaneous and safe administration of significant amounts of mapharsen, bismuth or both, sufficient to effect cure by virtue of their adjuvant or synergistic action with penicillin (3) in some of the cases who would otherwise not be cured by penicillin alone.

3. *Effect of an increase in dosage of penicillin:* a. An increase in the dosage of penicillin per injection would promote its therapeutic efficacy by virtue of the higher peak blood levels attained and the somewhat longer periods of time for which it would remain at demonstrable ("effective") levels after each injection. It has, however, been shown that the rate of spirocheticidal action of penicillin is not linearly proportional to its concentration (13), and that these high peaks represent largely waste penicillin (cf. page 180).

b. The larger dosage of penicillin would be more effective if it were applied, not as an increase in the amount administered per injection, but as an increased number of injections. Those additional injections could be given within the same total time period by increasing the fre-

quency of administration (e.g. every 2 hours instead of every four hours); but *the experimental data strongly suggest that they would be even more effective if the total duration of treatment were prolonged instead, and if the interval between injections were not reduced* (cf. pages 175–177, and figs. 2 and 3).

Present information indicates a relapse rate of approximately 15 to 25 per cent within 11 months in patients with early syphilis who had received a total of 1.2 to 2.4 million units of sodium penicillin in 8 days (60 injections of 20,000 or 40,000 units each at 3-hour intervals). In the light of the foregoing discussion, this could probably be appreciably reduced by an extension of the same treatment schedule, to a total time period of 16 days, and to a total dosage of 4.8 million units. Under such circumstances, the dosage, the duration of treatment and the number of injections are all doubled. Patients in whom e.g. 99.99 per cent of the organisms had been killed in 8 days might be effectively sterilized with continued treatment at the same dosage level per injection.

4. *Interval between injections:* Although a minimum interval must be allowed between injections in order to permit each dose of penicillin to exert its full effect, (cf. figs. 2 and 3) it is to be emphasized that this is a minimum interval, and not an optimum. In the rabbit, *the interval between injections can be prolonged far beyond that necessary minimum without affecting therapeutic activity.* Thus, for 16 injections given at 4-hour intervals, the CD_{50} was estimated to be 4000 units per kg.[4] When the interval between injections was further prolonged to 24 hours, the CD_{50} remained essentially unchanged (4000 units per kg. for 16 daily injections), with the suggestion that there may have been an optimum interval of 8–12 hours (CD_{50} of 1770 units/kg.).

The probable explanation is that after inadequate antisyphilitic treatment, the residual spirochetes multiply so slowly that even a 1-day interval between injections does not result in a significant interim increase in the number of organisms. In man, as in rabbits, relapse after inadequate treatment with either mapharsen or penicillin may not become manifest for weeks or months (cf. (8)), reflecting that slow multiplication. Equally significant, the curative dose of mapharsen in man is the same whether it is given over a period of 5 days, 3 times weekly for 10 weeks, or weekly for 20 to 30 weeks (cf. (10)), a finding

[4] Cf. footnote 1, page 172.

which is difficult to explain on any basis other than a slow multiplication in the interval between injections.

(It is interesting to note in this connection that in experimental trypanosomiasis of rats, in which there is a rapid and massive blood infection, Swinyard, Hirschfelder and Wright (15) have found the interval between injections of mapharsen to be of crucial significance; while in human trypanosomiasis, in which the organisms multiply only slowly, and are sometimes difficult to demonstrate even months after infection, large variations in the frequency of injection have had no demonstrable effect on the total curative dose of an active arsenical (16)).

At least in the treatment of experimental syphilis, and perhaps in the human disease as well, it is therefore not necessary that penicillin be given either regularly or as frequently as every 3 hours. In the rabbit, a given number of injections were about as effective whether given at intervals of 4 hours, 8 hours or daily, and were more effective so administered than if given too often, at e.g. one- or two-hourly intervals. If this is also true in man, treatment with penicillin need not necessarily be carried out at 3-hour intervals in hospitalized patients, but may be given on an ambulatory basis at infrequent or perhaps even irregular intervals without sacrificing therapeutic efficacy. The important therapeutic consideration is simply that the spirochetes be exposed to adequate tissue concentrations of penicillin for an adequate total period of time. The maximum interval which may be allowed between injections without impairing therapeutic efficacy will be determined in large measure by the degree to which the absorption and excretion of penicillin can be delayed.

5. *The "effective" blood level of penicillin:* The experimental data here presented, and the foregoing inferences drawn from those data, agree in the main with the searching analysis by McDermott, Benoit and Dubois (21) of the time-dose relationships in the penicillin treatment of human early syphilis. These workers have also concluded that peaks of high penicillin concentration contribute but little to therapeutic efficacy, and that its length of action at low concentration is the important factor. They further indicated the possibility that treatment need not be continuous to produce the maximum effect. The data here reported are in complete accordance with these conclusions.

They have, however, further suggested that the efficacy of a given

regime of treatment may be measured in terms of the total time for which it provides effectively spirocheticidal levels of e.g. 0.078 units per cc., that being the smallest amount capable of accurate determination by the particular technic of assay used. That time period was calculated for a variety of schedules in current use, and correlated with the available data with respect to the incidence of relapse. A number of treatment regimens were outlined which provide that effective level for periods of 90, 120 and 160 hours by a minimum number of intramuscular injections.

The present experiments, on the other hand, indicate that one cannot arbitrarily characterize any one concentration of penicillin as an "effective level", and ignore either the definitely greater activity per unit time of higher concentrations, or the equally definite, if slower, activity of lower concentrations. Extremely high concentrations acting for relatively short periods (e.g. 4 injections, at 4-hourly intervals, of 20,000 units per kg. each) and extremely low concentrations acting for long periods (50 injections, at 4-hourly intervals, of 7 mg./kg. each) were equally effective. Just how small a concentration of penicillin is capable of exerting a spirocheticidal action, and effecting cure if maintained for the necessary period of time (cf. page 175), remains to be determined. It is probably less than the 0.01 units per cc. which temporarily inhibits the growth of cultured S. pallida in vitro (13), since the penicillin in these cultures deteriorates under the conditions of the experiment. It is certainly a small fraction of 0.078 units per cc. In the most efficient schedule of treatment studied in the present experiments, all the animals tested were cured at dosages so small (10 units per kg. per injection) that penicillin was never demonstrable in the blood. There is reason to believe that 30 minutes after each injection at this dosage, the blood level was less than 0.005 units per cc. (i.e., less than 0.0003 mgs. per cent, or three parts per billion).

The therapeutic activity of penicillin is apparently a complex function of two variables, the tissue concentrations and the time over which they act. Although the latter is by far the more critical, the former must also be taken into consideration as a variable which affects the rate of spirocheticidal action, and thus, the therapeutic action of the drug.

6. *Applicability of the present discussion to diseases other than syphilis:* The degree to which the present data, and the conclusions drawn from

those data, are applicable to infections other than syphilis remains an open question. Whether a relatively low level sustained for a long period (many small injections) is more effective therapeutically than a high level for a short period (a few large injections) will depend in large part on the kinetics of the action of penicillin on the particular organism. In the case of spirochetes, it had been shown that the rate at which the organisms were killed did not increase in proportion to concentration (13). One could therefore have anticipated that a sustained low level would be more effective therapeutically than a temporarily high level, although the quantitative aspects of that relationship were surprising. With other organisms, however, data are not as yet available which permit an a priori estimate of this factor.

The same considerations apply also to the relative advantages of a) an increased dosage of penicillin per injection and b) an increased number of injections. Where bactericidal activity increases linearly with concentration, then both would be expected to be effective, and to approximately the same degree. However, if the rate of bactericidal action is less than linearly proportional to concentration (and this is the case with spirochetes), then an increase in the number of injections would be more effective than an increase in the size of the individual dose.

The optimum interval between injections of penicillin will be affected by several variables. One is the rate of the multiplication of organisms in the absence of penicillin. If that is rapid, then injections of penicillin must be given sufficiently often to maintain a level at which the organisms will not multiply. If, on the other hand, the organisms multiply slowly (e.g. spirochetes), then even a hiatus of 4 to 12 hours may not permit sufficient redissemination of organisms in the interval between injections to affect therapeutic efficacy. A second and related factor is the possibility that organisms damaged but not killed by penicillin may recover sufficiently in the interval between injections to require longer additional exposure to the drug than would otherwise have been the case.

SUMMARY

1. The therapeutic efficacy of sodium penicillin in experimental syphilis is profoundly modified by the method of its administration.

a. In rabbits injected at 4-hour intervals, as the number of injections

was increased from 8 to 20 to 50, and the duration of treatment from 32 hours to 8 days, the total curative dose (CD_{50}) fell from 80,000 to 1600 to 360 units per kg., respectively. Similarly, in rabbits injected twice daily, merely doubling the number of injections from 8 to 16, and the duration of treatment from 4 days to 8 days, reduced the total curative dose (CD_{50}) from 30,000 to 1770 units per kg.

b. When the number of injections was kept constant, and only the interval between them varied, the curative dose was of the same order of magnitude whether injections were given every 4 hours, twice daily or daily (4000, 1770 and 4000 units per kg.). On the other hand, when injections were given so frequently as to produce cumulative effects on the blood penicillin level, therapeutic efficacy was paradoxically reduced. Thus, the curative doses of sodium penicillin given in 16 injections at 4-hourly, 2-hourly and 1-hourly intervals were 4000, 32,000 and >64,000 units per kg., respectively.

c. When the total duration of treatment was fixed, and the number of injections varied, the therapeutic efficacy of penicillin increased with the frequency of injection. In rabbits treated over a period of 4 days, as the frequency of injection was changed from once daily to twice daily to every 4 hours, and the number of injections correspondingly increased from 4 to 8 to 20, the total curative dose (CD_{50}) fell from 50,000 to 20,000 to 1600 units/kg.

2. Although the therapeutic action of penicillin clearly involves both the tissue concentrations and the time over which they act, the latter time factor is by far the more important. Low concentrations acting over a long period of time (i.e., many small injections) were more effective than high concentrations acting over a short period (i.e., a few large injections to the same total dose). Within the time limits of the present experiments, the interval between injections was immaterial, provided they were not given too often: for an equal number of injections, treatments once daily were as effective as injections every 4 hours, with a suggestion of an optimum interval of 8 to 12 hours.

3. On the basis of the present experimental data, it may be anticipated that the results in the treatment of human early syphilis with sodium penicillin could be significantly improved by a) prolonging the duration of treatment, b) increasing the frequency and number of injections, and c) increasing the total dosage of penicillin. The relative importance of these three factors is discussed in the text. The use of

a suspension of calcium penicillin in oil and beeswax, or the administration of penicillin in a continuous intravenous, intramuscular or subcutaneous drip, or any other procedure which delays the absorption and excretion of penicillin would have the same effect as increasing the frequency and number of injections.

4. There is reason to believe that the treatment of syphilis with sodium penicillin need not be carried out in hospitalized patients, but that it may be given on an ambulatory basis once or twice daily, without necessarily sacrificing therapeutic efficacy, provided only that the patient receives the requisite total number of injections. The use of calcium penicillin in oil and beeswax, or of similar devices to delay the absorption and excretion of penicillin, may permit treatment to be given as infrequently as twice weekly, and perhaps even irregularly within that time interval.

5. The total curative dose (CD_{90}) of penicillin on the best schedule here tested was 500 units/kg. or approximately 0.3 mg./kg. of penicillin G. Milligram for milligram, penicillin is therefore 10 to 20 times as effective as mapharsen in the treatment of rabbit syphilis. In the human infection, however, penicillin is apparently only 2 to 4 times as active as mapharsen, mg. for mg.

With either penicillin or mapharsen, rabbit syphilis is easier to cure than the human disease, requiring less than $\frac{1}{4}$ to $\frac{1}{8}$ as much mapharsen, and less than $\frac{1}{20}$ to $\frac{1}{10}$ as much penicillin.

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PERFECTIONISM IN MEDICINE*

DR. RUFUS COLE

One morning, forty-five years ago, when I was an assistant in the Johns Hopkins Hospital, I received a telegram from Dr. Osler who was away on a vacation. The telegram read, "Please look in all the dictionaries for definitions of chauvinism and let me know what you find." I had then never heard of chauvinism or of Chauvin, but I did as I was asked. It later transpired that he was writing his well-known and beautiful address on Chauvinism in Medicine. In thus searching for all possible information on his subject, he was employing the methods of another "ism", namely, perfectionism. I have looked up this word for I suspected that I might be coining a new one, but I found that the theologians have been ahead of me. It is possible that their pretensions may be partly responsible for the odium now attached to all variants of this word. Moreover, the application of the term perfectionist to those who exhibit an extravagant meticulosity in unimportant details has become so common that considerable courage has been required to venture to discuss this subject, even in a graduation address, on which occasions a special tolerance is granted to the old in giving advice to the young, and when the speaker is not held to a strict accountability in practicing what he preaches.

Dr. Osler condemned chauvinism in no uncertain terms. I intend to try to convert you to the importance of perfectionism in medicine, or at least to persuade you not to treat it with contempt, or to ridicule those who practice it. It is merely an aim, a goal to be attempted, probably never to be reached, and its followers should be humble, not proud. The devotees of this cult must have imagination and the will and ability to work without ceasing, to take infinite pains. In other words, it demands of its followers imagination, determination and patience.

The habit of doing with completeness everything the hand finds to do is not perfectionism, though much can be said for it. That alone may be futile. The perfectionist must be able to decide what is important for him to be meticulous about. This requires more than a *little* knowledge. Years ago I knew a woman who had acquired a smattering

* Address to the graduating class of the Johns Hopkins University School of Medicine on March 19, 1946.

of bacteriology. She spent so much time in trying to keep bacteria out of her house that life for her family became unendurable. She would have no rugs, as they harbor bacteria; she kept the windows closed, because the air contains germs. This woman was not a true perfectionist. On the other hand, given knowledge and judgment, the imagination to set a goal and the will to reach it at whatever cost,—this constitutes perfectionism. It is to "dream, strive to do, and agonize to do."

It is sometimes said that perfectionists are born, not made. In spite of the pronouncement of the bard, it is doubtful if men have true greatness thrust upon them. Most men must achieve greatness, though a few may be born great. In recent years we have heard much loose talk about equality and freedom. There is no greater falsehood than that all men are born equal. As some lions are stronger than others, and some birds can fly higher, and some horses can run faster. so some men are more capable than others in one or another way. John Keats was born to sing, though it is true that he took great pains to improve his voice. When he was about the same age as many of you, he finished his hospital service, said good-bye to his fellows at Guy's and St. Thomas's and went away for a rest and a vacation. One morning, between dawn and ten o'clock, he composed his superb and immortal sonnet, "On first looking into Chapman's Homer." His perfectionism seems to have been inherent in his germ plasm. But such mutants are rare, and unfortunate is the man who conceives himself to have been born with special gifts, especially if he does not possess them.

No less misleading than the talk about equality are many of the views expressed about freedom. In most of the affairs of life we are not masters of our own destinies. We are largely what God, and our ancestors, and our environments have made us. And foolish is the man who boasts of his success. But whatever doubts we may have about freedom, even freedom of the will, there is one kind of freedom that cannot be denied us, that is freedom to try. We may share this freedom with the brutes, but at least we try consciously. Moreover, there is no limit to the heights we can attempt, and blessed is the man who can dream of heights never before attained.

> "A man's reach should exceed his grasp,
> Or what's a heaven for."

But merely dreaming dreams does not make a perfectionist, though it is very important. One doesn't reach the top of the mountain by merely dreaming about it. A short run and a jump don't cover much ground. A long period of training is required before the actual ascent is commenced, and to scale the heights he must mount slowly, paying careful attention to each step. It is especially the long and laborious preparation that tries the soul, but it is the willingness to undertake this that distinguishes the man of long view from the opportunist. One who hopes to reach his goal must listen to the words of the apostle, "Let patience have her perfect work, that ye may be perfect."

Some years ago, my portrait was painted by Cecelia Beaux, one of our greatest painters. Her pictures look as though they were made with long bold strokes, the hand being guided only by an unerring genius. To my surprise, I found that what appeared to be accomplished with such ease was only produced through the greatest care and attention to detail. To save my time it was suggested that a young man be employed to pose for the painting of the gown which I was wearing. At the end of the first session, he fainted. I was delighted to resume the sittings, for I learned very much from her about the importance of attention to details. When I later read her interesting autobiography, I learned of the long period of training and the labor and care that had been required to make her the matchless painter she was. Now I know what is wrong with many of the young impressionists who paint a dozen pictures in the time that she took to paint one and who have neglected preparation and scorned perfectionism.

While patience and care are important in art, they are still more important in science. Science, indeed, consists only in the slow accumulation of small facts in the hope of obtaining ultimate knowledge. Most of the scientific knowledge we possess was obtained through the painstaking efforts of perfectionists. And in no branch of science is perfectionism more important than in medicine.

Fortunately, I do not need to wander far from the precincts of this hospital and medical school to find examples of those who accepted this doctrine. It would be invidious to mention those still among us who belong in this group. But, in any case, my associations are largely with those who have lived and worked here in the past, many of whom have gone where aspirations as well as accomplishment are weighed in the balance and help to "swell the man's amount."

No better example of a man who embraced perfectionism can be given than the founder of the Johns Hopkins Hospital. In the letter to the trustees whom he chose to carry out his desires, he said, "it is my wish that the plan . . . shall provide a hospital which shall in structure and arrangement compare favorably with any other institution of like character in this country or in Europe." That means that he proposed to found a hospital that should excel any hospital then in existence. What a counsel of perfection that was. The amount of money which he left for this purpose was, even for those days, not enormous. Greater benefactions had been made before this for charitable and educational purposes, but probably never before had a similar gift been accompanied by such a vision of perfection. He did not ask for the largest hospital, what he emphasized was quality. He furthermore said, "It will be your especial duty to secure for the hospital, physicians and surgeons of the highest character and of the greatest skill." He said nothing about employing his family physician or those local doctors having the greatest reputation. He demanded the best to be obtained anywhere. Most people probably said at the time that the old man was in his dotage and was suffering from delusions of grandeur. Even today, does it not seem astounding that this supposedly hard-headed, practical business man, living in a relatively small conventional city, should have been such a perfectionist. It only shows how little we know of the personalities of our neighbors. Today, when we are fully aware of what his vision has meant to posterity, what an influence it has had on the progress of medicine, not only in this country but over the entire world, we realize what a wise man he was, and we wonder how any one can still have doubts about the importance of perfectionism.

The trustees that he appointed were faithful to their trust. They recognized that time would be required to carry out the large order, and they were patient. It was sixteen years after he wrote the letter to them, and probably many more after he first conceived the idea of founding a hospital that would be as near perfect as possible, that its doors were finally opened for the reception of patients. How much resistance the trustees must have exerted in opposing the demands of less far-seeing individuals who were impatient for immediate results. How the practical men of Baltimore must have pointed to the many poor people of the city who were in dire need of hospital care. The

trustees were undoubtedly accused of heartlessness, selfishness and pride. But, fortunately they could not be hurried. That they were men of vision is furthermore shown by the fact that, to assist them, they chose two perfectionists, John Shaw Billings and Daniel C. Gilman.

You have all heard how Dr. Billings visited all the best hospitals in this country and in Europe, picking out the best features of each one, and incorporating them in a plan which he evolved from his own great storehouse of knowledge. Nor did he refrain from drawing on his imagination in his attempt to devise the perfect hospital. Johns Hopkins may never have heard of Daedalus, but Dr. Billings was undoubtedly familiar with his warning.

> "My Icarus, he says; I warn thee fly
> Along the middle track: nor low nor high;
> If low, thy plumes may flag with ocean's spray;
> If high, the sun may dart his fiery ray."

But Billings, like Icarus, disdained this advice. Time has shown its fallaciousness. The modern Icarus need fear no danger if his wings are made of something more substantial than wax.

It is of interest that Dr. Billings presented two plans to the trustees. One of them was the ideal one, and he warned them that it would require a large amount of money, and would necessitate a considerable delay in opening the hospital. Under the other plan, much less money would suffice and the hospital could be opened very soon. But he advised that if the latter plan were adopted, the ideal plan of organization should be abandoned completely; there should be no compromise. "for if it is, the result will be satisfactory to nobody." Fortunately, the trustees followed his advice and adopted the ideal plan. In his later work, as head of the Surgeon General's Library and Director of the New York Public Library, Dr. Billings continued to show his great capacity for attention to details and his great powers of imagination. The Index Medicus, an ambitious project for making all medical literature readily available, involved an enormous amount of detailed work. It was a monumental achievement, second only to his planning the Johns Hopkins Hospital.

The second perfectionist that the trustees appointed to assist them

was Daniel C. Gilman. When the buildings were almost ready and the time for opening the hospital was approaching, the trustees asked Mr. Gilman, then president of the university, to take over for a time the additional burden of acting as superintendent of the hospital. It was the founder's wish that the hospital should ultimately form a part of the medical school of the university. Mr. Gilman was therefore the right man to make plans for its organization. In his previous work at Yale and at the University of California, he had shown that he was a man of far seeing vision. At Yale he had insisted that the Sheffield Scientific School should be a real school of science and not merely a technical school. He never thought of education in terms of brick and mortar, and it was largely through his efforts that the Johns Hopkins University became not merely another college. Incidentally, it may be remarked that as a result of his example and influence at least one of the presidents of the United States was a perfectionist. When Mr. Gilman took over the final steps in the organization of the hospital, he said he proposed to provide it with a system which should serve as a guide for other institutions. In installing this system he overlooked no details. He visited not only other hospitals, but also hotels, where he studied the details of management, inspecting kitchens, laundries and linen rooms. He even investigated such minor matters as table linens and napkins.

In choosing men to staff the hospital and to become members of the faculty, the same meticulous care was exercised. As a result, there developed an atmosphere of perfectionism that pervaded everything connected with the institution. All this had a tremendous influence on the young men who came here later to enter the medical school. For those with high ambitions, the long years of preparation are generally held to be difficult ones. It is hard to be patient. But when ispired by imagination, this period of training and preparation, instead of being monotonous drudgery, may be a time of the greatest joy and happiness. When I came here and became a member of one of the earliest classes, I entered a new world, a world in which everyone seemed to be inspired by visions and to be working with feverish intensity to prepare himself for sharing in the great new developments in medicine which the new hospital and the new school seemed to promise. There were then only sixty or seventy students in the

school, all of them college graduates, and in those days a college degree conferred at least a moderate amount of distinction. But most of them, even those coming from the best colleges, apparently realized for the first time the importance of working for the highest objectives; they became serious students, not merely carrying out tasks set for them. They all seemed animated by a desire to master all that was known about man and his ailments. The professors made little or no effort to urge them to work. The example of the perfectionists who founded and organized the school and of those who were then directing its activities, seemed to be a sufficient stimulus. They had all seen a vision. It was surprising how happy they all seemed in the unremitting and arduous toil of preparation. The explanation is that they had all been converted to perfectionism. Moreover, a fellowship developed among these disciples like that which is present among the followers of a religion. There was little or no envy or spirit of rivalry, nor was there evident any undue ambition for future personal success. For the present, the work was all absorbing.

A large group of the students then lived in the houses facing Jackson Square. After working all day in the hospital and laboratories, they had dinner in the nearby boarding houses, and then, especially on pleasant evenings, they were accustomed to congregate about the fountain which stood in the middle of the Square. There they engaged for a short time in gossip and banter, and in conversation, some of it high, some of it not so high. But there existed among them an indiscribable spirit of high endeavor. There was an atmosphere of otherworldliness like that which must have been present in the monasteries of old. One was conscious of the other world across town, with its pleasures and distractions, but for the time being that other world seemed distant and unreal. There were then no movies to attend, no automobiles to carry one quickly into the midst of bright lights and gaiety. These interludes did not last long. Soon, one by one, the men would depart. Like horses, eager for the race, they hastened to get back to their studies, and lights would appear here and there in the windows overlooking the Square, and one knew that behind each one was a student eagerly trying to acquire the knowledge that had been accumulated by the workers of the past.

The recollection of Jackson Square reminds me of Robert Louis

Stevenson's account of the Forest of Fontainebleau, and of Barbizon. For many years young artists have lived within the shades of this smiling forest and have there passed their period of apprenticeship, perfecting their techniques, painting studies and school pictures, and dreaming dreams. For the time, Paris, with all its distractions, though only a few miles away, might as well have been on another planet. Stevenson says, "There are few things more renovating than to leave Paris, the lamplit arches of the Carrousel, and the long alignment of the glittering streets, and to bathe the senses in the fragrant darkness of the wood .... Here one might forget that there existed other and more pressing interests than those of art .... We were all artists, almost in the age of illusion, cultivating an imaginary genius, and walking to the strains of some deceiving Ariel; small wonder, indeed, if we were happy."

It is a far cry from "the majestic avenues of highways, the wilderness of tumbled boulders, the great age and dignity of certain groves" of Fontainebleau, to the patchy lawns, the simple fountain, and the austere brick houses of Jackson Square of fifty years ago, but the atmosphere with which they were both pervaded, and upon which the charming, mysterious quality of both largely depended, was not due to the trees or bricks, but to the spirit of the inhabitants. It is true that some of the quality of Fontainebleau was related to the memories of the great artists, like Millet and Rousseau, who had lived and worked there. The young men of Jackson Square received no inspiration from departed inhabitants; they were stimulated only by the example of the talented and industrious teachers who were working under the new brick dome which was visible over the roofs of the neighboring houses.

The rare atmosphere of perfectionism, however, is not able to sustain either artists or medical students for twenty-four hours every day in the week, and it must be confessed that the charming Siron's Inn at Barbizon, which Stevenson describes so entertainingly, had its counterpart in the little saloon around the corner, where on Saturday nights one might find the back room filled with the high-minded youths drinking beer and playing poker for small stakes. I am afraid that if I confess to you the kind of picture that this modest place of refreshment calls up, you will at once decide that my whole memory of

Jackson Square is but the illusion of an old man wearing rose-tinted spectacles. Nevertheless, I must say that no club I have ever visited has seemed so glamorous, no host so genial, no wit so sparkling, no excitement so stimulating, no companionship so pleasant as that present in the little back room, with its sawdust covered floor and its delightful odor of mixed smoke and beer. It may all be an illusion, but it is no illusion that the group of hard working students in Jackson Square were all happy.

The fountain in Jackson Square exists no more, and most of the disciples of perfectionism who gathered about it in the twilight have also departed. It is possible, however, that on pleasant spring evenings, medical students living in that neighborhood, may encounter the shades of some of them whose names are permanently written in medical history, wandering about, anxiously searching for the lost fountain. Whether later disciples of this creed have found other retreats of peace and quiet in which to practice their religion, I do not know. In the midst of all the noise and confusion and restlessness and distractions they may have decided that the need for sacred groves and altars no longer exists. In some places it has been thought that only lofty temples made of marble and stone and containing a labyrinth of apartments and busy workshops are fit places in which to worship.

One sometimes wonders whether the enthusiasm existing in a young institution, starting in a dream of glory and surrounded by a halo of perfectionism, can ever be entirely preserved. Some years ago, when I was experiencing this fear about another institution with which I was associated from its inception, I rather naïvely remarked to the late President Lowell of Harvard that the first years of many institutions seemed to be the best ones. He replied by asking me if I thought it would be advisable for Harvard to close its doors and another university be started. In any case, at the Johns Hopkins Hospital and Medical School the perfectionism did not disappear after the first wave of enthusiasm. There have always been many perfectionists among the members of the faculty. Among them there has probably never been a better example of a true perfectionist than Dr. Halsted. It was difficult, even for his students, fully to realize the importance of his meticulous, painstaking work. The care and attention which he gave to the most minute details in his operations failed to impress them

like the brilliant strokes of other dexterous operators. Yet it was through his work that success was achieved in the operation for cancer of the breast and in suturing the intestine. It is only in later years that the realization has come that this most meticulous of men exerted an influence on surgery greater than that of any other surgeon of his time. One can forgive such a man for sending his shirts to Paris to be laundered.

I may mention one other example of the important results arising from extreme attention to details. I well remember going to see an autopsy early one morning, and after giving a few hasty glances, I hurried about my daily tasks, somewhat impatient with the slowness of the young pathologist who was performing the autopsy. Late in the afternoon, when the lights were growing dim, I groped my way through the old dark corridor and, passing the autopsy room, I peeped in. There was the young pathologist still searching and studying the material. As a result of that autopsy, there developed one of the most important discoveries that has emanated from this institution.

It is very easy to be superficial and careless. This is sometimes the result of insufficient preparation and lack of appreciation of the task one sets himself. When I was working in the research clinical laboratory, one day a young student came to me and said that he expected to have a little free time, a few hours a week, and that he would like to undertake a small research. I asked him if he had thought about what he would like to do. He replied that he thought he would like to find the cause of measles. At this time the etiology of this malady was completely unknown. I felt like asking him if he would be willing to spend his life in preparation and in carrying out his project. However, I merely suggested that he first undertake some less ambitious problem. He had the dream but no comprehension of what would be required to realize it. Not that dreams are unimportant, and woe be to him that would smother them. They are requisite for the perfectionist, but alone they are not sufficient.

More often, superficiality and carelessness result from attempting too many things, from spreading out too thin. Medical schools are not entirely without blame for this, but they have had to yield to much outside pressure. To expect students to acquire all the knowledge that has been accumulated over centuries is to ask the impossible. I shall not suggest how this can be changed, but will merely say that, in

my opinion, it is more important that the doctor should be trained to do his work thoroughly than that he should have encyclopedic knowledge. In other words, medical schools will do well to produce perfectionists.

Those of you who during your college and university years have embraced perfectionism and will continue in scientific and educational work will encounter no great difficulties in remaining true to your creed. On the other hand, those who will soon become separated from their fellow disciples and engage in private practice will enter a world where perfectionism is frequently ridiculed and derided. You will find it more difficult to continue to be painstaking and meticulous and to practice what the "world's coarse thumb and finger failed to plumb." I have a friend of advanced years who recently suffered from a most troublesome ailment. He, himself, thought he had one foot in the grave. He consulted a very busy, and very successful doctor, one who had received as good a training as that which you have had. This doctor instituted a course of treatment, which was continued for a long time, but the patient became no better. When he complained to the doctor, the latter replied, "Oh, you are a perfectionist", thereby implying that my friend should be content to spend the remainder of his life hopping along on one leg and with the other dragging his grave after him. Somewhat annoyed, he consulted another physician. This new doctor continued the same treatment, but he exercised the greatest care in the way it was administered, and my friend was soon perfectly well. Because of this lack of care on the part of many doctors, the practical results following the application of the great discoveries in medicine of recent decades are falling far short of what might be expected of them. Not only do the wonderful new drugs often fail in their action but they may be actually harmful. So too, operations are not infrequently so hastily performed and without sufficient attention to details that they are only halfway successful or are failures.

With the increasing tempo of life, the desire for immediate recognition and rewards on the part of the doctor grows accordingly. If the young doctor is willing to employ a suitable technique, these rewards can often be obtained sooner than is good for him. A man who yields to this temptation is soon darting about hither and yon, making a great show of business. He soon employs technicians and assistants

to do the work that he was trained to do and which he ought to do himself, if it is worth doing at all. He joins a large number of medical societies, the scientific meetings of which he rarely attends. He does nothing completely, and seeks a reputation for skill and brilliancy which he usually does not possess. The undeserved success of men of this type is often blamed on the public. But people are more discriminating than is generally believed and usually in time come to recognize real ability and appreciate care and thoroughness. I sincerely believe that if you will make a practice of treating every patient with the same care and thoroughness that the true artist devotes to every picture, each one of which he regards as a possible masterpiece, you will sooner or later receive recognition, not only from your colleagues but from the public as well. In my experience, it is the perfectionists who become the consultants.

An exaggerated emphasis on material rewards on the part of doctors is in part responsible for the present trend toward organization of the profession, and this leads to standardization which results in a glorification of mediocrity. The most serious calamity that can happen to the young doctor is for him to become obsessed with the idea that he is endowed with the qualities of leadership and become involved in medical politics and spend useless hours in committee meetings and in making plans to organize the profession. So far no attempt has been made to organize the perfectionists, but even this may happen.

Recently a serious discussion has arisen concerning the time required by a doctor to examine and treat a patient; how many patients can be passed through his office in an hour; how fast should his assembly line move. The Florentines might as well have discussed how many angels Fra Angelico could paint in a day, in order to set a time standard for angel painting.

The best advice I can offer to the young physician is not to be afraid of being called a prima donna or a perfectionist. Operas will be dreary affairs when there are no prima donnas, and medicine will become sterile when no doctors are perfectionists. Fortunate is the young doctor who possesses what has been called "a passion for perfection."

However, there is one warning that I should utter. Practice perfectionsim, but don't talk too much about it. Leave that to the old doctors who have tried, and are no longer concerned about their failures.

A LETTER TO THE EDITOR

"And it came to pass in those days. . . ." Memories were awakened when, among some old records, I discovered the electrocardiogram of one Marcellus Holmes, who may be recalled by the House Staff and the students of thirty years ago. Back in those days, when Theodore Janeway was our "Chief," when William Welch mixed philosophy with pathology, when Thayer and Barker bewildered us with their brilliant clinical expositions, when Mosenthal, Austrian, Bloomfield, Bridgeman, Guthrie and Sydenstricker led the group of medical assistants and associates, it so happened that another character entered. He was Marcellus Holmes, a gentleman of uncertain age and habits, and of a still more uncertain social status, but whose name should be recorded in the Annals of The Johns Hopkins Hospital, as a frequent and generous contributor to scientific knowledge.

As the chilly dampness of each Baltimore winter approached, Marcellus would recall the pleasant warmth and comfortable quarters which he had previously enjoyed in the hospital wards, and the genuine cordiality with which he was annually greeted by instructors and students alike. His motives, if I mistake not, included a desire for regular nourishment and relief from the responsibilities which rested heavily on his soul. By 1916 he already knew the pattern of approach—a short wait in the reception room, assignment to a student examiner, a stripping off of coat and shirt and then the customary inquisition. The stethoscope was to Marcellus a key to the solution of all his problems—first in the hands of the student, then of the instructor and the attending physician, after which he well knew would come the assignment to a medical ward for "observation." The popularity of Marcellus was established from the moment of his admission. Having had the same experience for many years, his behavior as a subject of clinical study and investigation was perfect. He knew the meaning, in his own way, of various murmurs and pulsations. He would make suggestions to the amateurs who clustered about, as to where this or that physical sign could best be elicited. His blood pressure? "Yassuh, yassuh, a little high suh, 190 last year suh, 190 over nuthin. And the pistol shot, suh, that's what they calls

it, down thar suh." Murmurs? "Yassuh, up here suh, dats whar dey genally gets 'em." Thus went his conversation.

The incident which I am reporting occurred during March of 1916 in the old and dismal cardiographic laboratory, down at the end of a long basement corridor, to which subjects for investgation were taken. The original electrocardiograph was made by Mr. Hindle of New York under the personal supervision of Dr. H. B. Williams of Columbia, and was the third of its kind built in the United States. The

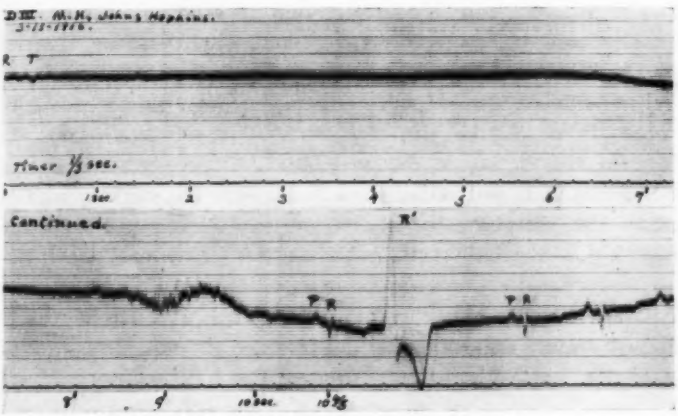

FIG. 1. Electrocardiogram of complete auricular and ventricular standstill for 10⅘ seconds resulting from simultaneous pressure on both vagi. Record taken at the old heart station of Johns Hopkins Hospital in March, 1916.

massive galvanometer was mounted on a cement foundation to eliminate building vibrations. A new control box with perhaps a dozen dials had just been installed and was the pride of the department. Film was used and developed in a long, five foot wooden trough in the adjoining dark room and prints were made from sections of this film. The heart station was also the proud possessor of a new roll film recording camera, which replaced the sliding plate of the still older Cambridge machine. This recording mechanism was placed some seven or eight feet from the galvanometer to provide the necessary amplification. The cracker jar terminals were still there, but only as

relics. They had been replaced by large electrodes, eight inches square, which were attached to the extremities over pads of salt-soaked gauze, and held in place by salt-soaked bandages. Such was our equipment.

Now at the time of Marcellus' visit to the Heart Station, the subject of vagus influence on the rate and rhythm of the heart was to us the most important in medicine, and here was a reasonable and tractable subject from whom graphic records of the effects of carotid sinus pressure could be secured. Marcellus, on the table, was the center of our scientific interest. In this setting, with all attachments and adjustments in order, pressure was made on the right vagus. The heart slowed. We, Dr. Henry R. Carter, Jr. whom many will recall and I, tried pressure on the left side. Again the heart slowed and a little irregularity developed. An idea came to us simultaneously. Why not press on both vagi at the same time? Double carotid sinus pressure was made, the string fluttered, the camera was started, but to our dismay the movement of the string shadow stopped completely. Pressure was released but still no pulsation. Camera and timer ticked on. Marcellus twitched spasmodically. Perspiration stood out on Dr. Carter's face. Marcellus blinked his eyes. We felt that heaven was just around the corner for Marcellus—but not for us. Henry ventured a question as to how he felt—"Just a little dizzy suh, just a little dizzy." When the future seemed darkest for all three of us, the shadow of the string in the bright beam of light on the camera suddenly jumped across the field and soon settled down to its good old rhythm and the swinging motion was a beautiful sight for two frightened young medics. Our reputations were saved, and so was the life of Marcellus, who continued for many years to return each winter for his free bed and board, quite unaware of his one time close approach to the golden gates.

James Mackenzie mentions a complete standstill for from three to four seconds as a result of heavy digitalization, and Thomas Lewis refers to a case reported by Laslett, who tells of complete standsill of from four to eight seconds under vagal stimulation. Levine, of Peter Bent Brigham, shows a record of complete standstill for six and one-fourth seconds after left carotid sinus pressure. None of these have quite reached the record of Marcellus, who provided an electro-

cardiogram showing complete auricular and ventricular asystole for a period of ten and four fifths seconds.

Perhaps your readers will be interested in a copy of the electrocardiogram, and this little yarn about the "Old Heart Station," when electrocardiography was young.

Sincerely yours,
N. WORTH BROWN, M.D.

The Toledo Clinic,
Toledo, Ohio,
March 26, 1946.